STIC-ILL

Apr 1.57

From: Huynh, Phuong N.
Sent: Sunday, December 22, 2002 12:53 PM
To: STIC-ILL
Subject: RE: 09/865,198 Rush

Importance: High

Please deliver the following:

J Immunological Methods 230(1-2): 159-71; 1999

J Immunological Methods 267(2): 213-226; 2002

Cancer Research 61(19): 7002-8; 2001

~~J Biochemistry~~ 273(5): 2858-65; 1998 Journal of Biological Chemistry

Protein Engineering 13(5): 361-7; 2000

Anticancer research 19(2C): 1525-8; 1999

FEBS Letters 422(2): 259-64; 1998

FEBS Letters 432(1-2): 45-9; 1998

Current Medicinal Chemistry 3/2: 87-100; 1996

Nature Biotechnology 15(2): 159-63; 1997

J Immunology 162(11): 6589-95; 1999

J Immunological Methods 231(1-2): 177-189; 1999

International Immunology 10(12): 1863-72; 1998

Molecular Immunology 33(2): 211-9; 1996

Structure 2(12): 1217-26; 1994

Protein Engineering 7(8): 1017-26; 1994

International journal of Cancer 62(3): 319-24; 1995

Thanks,
Neon
Art unit 1644
Mail 9E12
Tel 308-4844

82964

Schreiber, David

From: Huynh, Phuong N.
Sent: Sunday, December 22, 2002 11:27 AM
To: Schreiber, David
Subject: RE: 09/865,198

Importance: High

David,

Please search peptides of SEQ ID NO: 2-8 and 21-23 against commercial and interference database.

Thanks,
Neon
Art unit 1644
Mail 9E12
Tel 308-4844

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NEWS 1		Web Page URLs for STN Seminar Schedule - N. America
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NEWS 4	Apr 09	ZDB will be removed from STN
NEWS 5	Apr 19	US Patent Applications available in IFICDB, IFIPAT, and IFIUDB
NEWS 6	Apr 22	Records from IP.com available in CAPLUS, HCAPLUS, and ZCAPLUS
NEWS 7	Apr 22	BIOSIS Gene Names now available in TOXCENTER
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NEWS 11	Jun 10	PCTFULL has been reloaded
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NEWS 19	Aug 19	Aquatic Toxicity Information Retrieval (AQUIRE) now available on STN
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NEWS 22	Aug 26	Sequence searching in REGISTRY enhanced
NEWS 23	Sep 03	JAPIO has been reloaded and enhanced
NEWS 24	Sep 16	Experimental properties added to the REGISTRY file
NEWS 25	Sep 16	Indexing added to some pre-1967 records in CA/CAPLUS
NEWS 26	Sep 16	CA Section Thesaurus available in CAPLUS and CA
NEWS 27	Oct 01	CASREACT Enriched with Reactions from 1907 to 1985
NEWS 28	Oct 21	EVENTLINE has been reloaded
NEWS 29	Oct 24	BEILSTEIN adds new search fields
NEWS 30	Oct 24	Nutraceuticals International (NUTRACEUT) now available on STN
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NEWS 33	Nov 25	More calculated properties added to REGISTRY
NEWS 34	Dec 02	TIBKAT will be removed from STN
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NEWS 38	Dec 17	Adis Clinical Trials Insight now available on STN

NEWS EXPRESS	October 14 CURRENT WINDOWS VERSION IS V6.01, CURRENT MACINTOSH VERSION IS V6.0a(ENG) AND V6.0Ja(JP), AND CURRENT DISCOVER FILE IS DATED 01 OCTOBER 2002
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FILE 'HOME' ENTERED AT 11:28:17 ON 22 DEC 2002

=> file medline embase biosis scisearch caplus

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.21	0.21

FILE 'MEDLINE' ENTERED AT 11:28:29 ON 22 DEC 2002

FILE 'EMBASE' ENTERED AT 11:28:29 ON 22 DEC 2002

FILE 'BIOSIS' ENTERED AT 11:28:29 ON 22 DEC 2002

FILE 'SCISEARCH' ENTERED AT 11:28:29 ON 22 DEC 2002

FILE 'CAPLUS' ENTERED AT 11:28:29 ON 22 DEC 2002

=> s bispecific polypeptide
L1 1 BISPECIFIC POLYPEPTIDE

=> d l1 cbib abs

L1 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS
1998:392226 Document No. 129:72204 Bispecific reagents for redirected targeting of human lipoproteins. Fanger, Michael W.; Morganelli, Peter M. (USA). U.S. US 5762930 A 19980609, 16 pp., Cont.-in-part of U. S. Ser. No. 955,681, abandoned. (English). CODEN: USXXAM. APPLICATION: US 1993-155114 19931119. PRIORITY: US 1992-955681 19921002.

AB Bispecific mols. (BSM) which react both with an Fc.gamma. receptor for IgG of human effector cells and with either human LDL (or a fragment thereof) or human HDL (or a fragment thereof) are disclosed. The BSM, which may be bispecific antibodies, heteroantibodies, or single-chain **bispecific polypeptides**, bind to an Fc.gamma. receptor without being blocked by the binding of IgG to the same receptor. The BSM having a binding specificity for human LDL are useful for targeting human effector cells (e.g. monocytes, macrophages) for degrdn. of LDL in vivo. The BSM with binding specificity for human HDL are useful for targeting human HDL to human effector cells such that the HDL takes up cholesterol from the effector cells. The BSM are useful for treatment and diagnosis of atherosclerosis.

=> s bispecific and VEGF receptor
L2 4 BISPECIFIC AND VEGF RECEPTOR

=> dup remove l2
PROCESSING COMPLETED FOR L2
L3 4 DUP REMOVE L2 (0 DUPLICATES REMOVED)

=> d l3 1-4 cbib abs

L3 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2002 ACS
2002:601462 Document No. 137:215471 Fab-scFv fusion protein: an efficient approach to production of **bispecific** antibody fragments. Lu, Dan; Jimenez, Xenia; Zhang, Haifan; Bohlen, Peter; Witte, Larry; Zhu, Zhenping (Department of Antibody Technology, ImClone Systems Incorporated, New York, NY, 10014, USA). Journal of Immunological Methods, 267(2), 213-226 (English) 2002. CODEN: JIMMBG. ISSN: 0022-1759. Publisher: Elsevier Science B.V..
AB The clin. development of **bispecific** antibodies (BsAb) as therapeutics has been hampered by the difficulty in prepg. the materials in sufficient quantity and quality by traditional methods. Here, the authors describe an efficient approach for the prodn. of a novel **bispecific** antibody fragment by genetically fusing a single-chain Fv (scFv) to the C-terminus of either the light chain or the heavy chain of a Fab fragment of different antigen-binding specificity. The **bispecific** Fab-scFv fragments were expressed in a single Escherichia coli host and purified to homogeneity by a one-step affinity chromatog. Two different versions of the **bispecific** Fab-scFv fragments were constructed using two antibodies directed against the two tyrosine kinase receptors of vascular endothelial growth factor. These **bispecific** antibody fragments not only retained the antigen-binding capacity of each of the parent antibodies, but also are capable of binding to both targets simultaneously as demonstrated by a crosslinking ELISA. Further, the **bispecific** antibodies were comparable to their parent antibodies in their potency in blocking ligand binding to the receptors and in inhibiting ligand-induced biol. activities. This design for BsAb fragments should be applicable to any pair of antigen specificities.

L3 ANSWER 2 OF 4 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2002:504980 Document No.: PREV200200504980. Intraperitoneal **bispecific** antibody (HEA125xOKT3) therapy inhibits malignant ascites production in advanced ovarian carcinoma. Marme, Alexander; Strauss, Gudrun; Bastert, Gunther; Grischke, Eva-Maria; Moldenhauer, Gerhard (1). (1) Department of Molecular Immunology, German Cancer Research Center, Im Neuenheimer Feld 280, 69120, Heidelberg: g.moldenhauer@dkfz:de Germany. International Journal of Cancer, (10 September, 2002) Vol. 101, No. 2, pp. 183-189. print. ISSN: 0020-7136. Language: English.
AB **Bispecific** antibody HEA125xOKT3 was shown to redirect T lymphocytes toward carcinoma cells and to induce tumor cell lysis in vitro. Preclinical studies have demonstrated that tumor-associated lymphocytes (TAL) derived from malignant ascites can be used as effector cells with a high efficacy and without prior stimulation. These data provided the rationale for a clinical trial to investigate whether bsAb HEA125xOKT3 is also able to induce tumor cell lysis in vivo and can be used for local treatment of malignant ascites arising from ovarian carcinoma. Ten ovarian carcinoma patients presenting with malignant ascites resistant to chemotherapy were enrolled in the study. They received weekly intraperitoneal injections of 1 mg bsAb diluted in 500 ml NaCl solution to allow homogeneous antibody distribution within the peritoneal cavity. All patients responded clinically well to the therapy. Eight patients experienced a complete and 2 patients a partial reduction

of ascites production. A decrease or stabilization of tumor marker CA125 was detected in the sera of 8 patients. Only WHO Grade I and II toxicity was observed including mild fever, chills and allergic eczema. Thus, intraperitoneal application of bsAb HEA125xOKT3 appears to be an easy and cost effective palliative treatment for ovarian carcinoma with recurrent ascites that leads to a substantially increased quality of life for the patients. During therapy TNF-alpha concentrations raised markedly in the ascites fluid whereas VEGF and sFLT-I ascites levels declined. This indicates that not only T cell-mediated tumor cell lysis but also changes in vascular permeability due to downregulation of VEGF and its receptors might be responsible for the beneficial therapeutic effect.

L3 ANSWER 3 OF 4 MEDLINE

2001538782 Document Number: 21469635. PubMed ID: 11585724. Complete inhibition of vascular endothelial growth factor (VEGF) activities with a bifunctional diabody directed against both VEGF kinase receptors, fms-like tyrosine kinase receptor and kinase insert domain-containing receptor. Lu D; Jimenez X; Zhang H; Wu Y; Bohlen P; Witte L; Zhu Z. (Department of Molecular and Cell Biology, ImClone Systems Inc., New York, New York 10014, USA.) CANCER RESEARCH, (2001 Oct 1) 61 (19) 7002-8. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Vascular endothelial growth factor (VEGF) binds to and mediates its activity mainly through two tyrosine kinase receptors, **VEGF receptor** 1 [or fms-like tyrosine kinase receptor (Flt-1)] and **VEGF receptor** 2 [or kinase insert domain-containing receptor (KDR)]. Numerous studies have shown that overexpression of VEGF and its receptor plays an important role in tumor-associated angiogenesis and hence in both tumor growth and metastasis. We demonstrated previously that antagonistic antibodies to KDR specifically inhibited VEGF-stimulated receptor activation, cell migration, and endothelial cell mitogenesis. Here we constructed a recombinant bifunctional diabody that is capable of blocking both Flt-1 and KDR from binding to their ligands, including VEGF and placenta growth factor (PlGF). The diabody was expressed in Escherichia coli and purified by single-step affinity chromatography. The diabody retained the capacity to bind both KDR and Flt-1 and effectively blocked interaction between KDR and VEGF, Flt-1 and VEGF, and Flt-1 and PlGF. Furthermore, the diabody is a stronger inhibitor than its parent antibodies to VEGF-stimulated mitogenesis of human endothelial cells, as well as both VEGF- and PlGF-induced migration of human leukemia cells. Taken together, our results suggest that dual receptor blockade with the bifunctional diabody may prove to be a more efficient approach in inhibiting VEGF-stimulated angiogenesis.

L3 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2002 ACS

1999:778461 Document No. 132:103296 Acquired antagonistic activity of a **bispecific** diabody directed against two different epitopes on vascular endothelial growth factor receptor 2. Lu, D.; Kotanides, H.; Jimenez, X.; Zhou, Q.; Persaud, K.; Bohlen, P.; Witte, L.; Zhu, Z. (Department of Molecular and Cell Biology, ImClone Systems, New York, NY, USA). Journal of Immunological Methods, 230(1-2), 159-171 (English) 1999. CODEN: JIMMBG. ISSN: 0022-1759. Publisher: Elsevier Science B.V..

AB **Bispecific** antibody (BsAb) technol. has been successfully used as a means to construct novel antibody (Ab) mols. with increased avidity for binding, by combining two Ab or their fragments directed against different epitopes within the same antigen. Using two single chain antibodies (scFv) isolated from a phage display library, the authors have constructed a **bispecific** diabody directed against two different epitopes on the extracellular domain (ECD) of human vascular endothelial growth factor receptor 2 (VEGFR2), the kinase-insert domain-contg. receptor (KDR). Neither of the parent scFv blocks KDR/VEGF interactions or inhibits VEGF-induced receptor activation. The diabody binds to KDR with an affinity that is 1.5- to 3-fold higher than its parent scFv, mainly due to a much slower dissocn. rate (koff), which is approx. 17- to

26-fold slower than that of the individual scFv. In addn., the diabody binds simultaneously to, and thus crosslinks, the two epitopes on the receptor(s). It is rather unexpected that the diabody effectively blocked KDR/VEGF interactions, and inhibited both VEGF-induced activation of the receptor and mitogenesis of human endothelial cells. Taken together, the authors' results suggest that the diabody is most likely to exert its effect through steric hindrance and/or causing major conformational changes of the receptor. This is the first report on the construction of a **bispecific** diabody with acquired novel antagonistic activity.

=> s bispecific and cytokine
L4 541 BISPECIFIC AND CYTOKINE

=> s l4 and single chain Fv
L5 10 L4 AND SINGLE CHAIN FV

=> dup remove l5
PROCESSING COMPLETED FOR L5
L6 3 DUP REMOVE L5 (7 DUPLICATES REMOVED)

=> d l6 1-3 cbib abs

L6 ANSWER 1 OF 3 MEDLINE DUPLICATE 1
2001448611 Document Number: 21240674. PubMed ID: 11342630. Increasing the affinity for tumor antigen enhances **bispecific** antibody cytotoxicity. McCall A M; Shahied L; Amoroso A R; Horak E M; Simmons H H; Nielson U; Adams G P; Schier R; Marks J D; Weiner L M. (Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA 19111, USA.) JOURNAL OF IMMUNOLOGY, (2001 May 15) 166 (10) 6112-7. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.
AB We tested the hypothesis that **bispecific** Abs (Bsab) with increased binding affinity for tumor Ags augment retargeted antitumor cytotoxicity. We report that an increase in the affinity of Bsab for the HER2/neu Ag correlates with an increase in the ability of the Bsab to promote retargeted cytotoxicity against HER2/neu-positive cell lines. A series of anti-HER2/neu extracellular domain-directed **single-chain Fv** fragments (scFv), ranging in affinity for HER2/neu from 10(-7) to 10(-11) M, were fused to the phage display-derived NM3E2 human scFV: NM3E2 associates with the extracellular domain of human FcgammaRIII (CD16). The resulting series of Bsab promoted cytotoxicity of SKOV3 human ovarian carcinoma cells overexpressing HER2/neu by human PBMC preparations containing CD16-positive NK cells. The affinity for HER2/neu clearly influenced the ability of the Bsab to promote cytotoxicity of (51)Cr-labeled SKOV3 cells. Lysis was 6.5% with an anti-HER2/neu K(D) = 1.7 x 10(-7) M, 14.5% with K(D) = 5.7 x 10(-9) M, and 21.3% with K(D) = 1.7 x 10(-10) M at 50:1 E:T ratios. These scFv-based Bsab did not cross-link receptors and induce leukocyte calcium mobilization in the absence of tumor cell engagement. Thus, these novel Bsab structures should not induce the dose-limiting **cytokine** release syndromes that have been observed in clinical trials with intact IgG BSAB: Additional manipulations in Bsab structure that improve selective tumor retention or facilitate the ability of Bsab to selectively cross-link tumor and effector cells at tumor sites should further improve the utility of this therapeutic strategy.

L6 ANSWER 2 OF 3 SCISEARCH COPYRIGHT 2002 ISI (R)
2001:825009 The Genuine Article (R) Number: 480NH. Specific immunotherapy of cancer in elderly patients. Matzku S; Zoller M (Reprint). German Canc Res Ctr, Dept Tumor Progress & Immune Def, Neuenheimer Feld 280, D-69120 Heidelberg, Germany (Reprint); German Canc Res Ctr, Dept Tumor Progress & Immune Def, D-69120 Heidelberg, Germany; Merck KGaA, Dept Oncol Biomed Res, Darmstadt, Germany; Univ Karlsruhe, Dept Appl Genet, Karlsruhe,

indirect immunofluorescence. H1 did not neutralize human IL-6, and the H1 epitope was mapped to a region of IL-6 not involved in interactions with IL-6, IL-6 receptor, or the signal-transducing protein gp130. To target IL-6-secreting cells, we then constructed a **bispecific** antibody fragment (a diabody) comprising H1 and the antigen binding site of the T-cell activating monoclonal antibody OKT3. The diabody led to T-cell-mediated killing of cells secreting IL-6.

=> d his

(FILE 'HOME' ENTERED AT 11:28:17 ON 22 DEC 2002)

FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 11:28:29 ON 22 DEC 2002

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L1             1 S BISPECIFIC POLYPEPTIDE
L2             4 S BISPECIFIC AND VEGF RECEPTOR
L3             4 DUP REMOVE L2 (0 DUPLICATES REMOVED)
L4           541 S BISPECIFIC AND CYTOKINE
L5            10 S L4 AND SINGLE CHAIN FV
L6             3 DUP REMOVE L5 (7 DUPLICATES REMOVED)

=> s dup remove l4
MISSING OPERATOR REMOVE L4
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> dup remove l4
PROCESSING COMPLETED FOR L4
L7           279 DUP REMOVE L4 (262 DUPLICATES REMOVED)

=> s l7 cytokine receptor
MISSING OPERATOR L7 CYTOKINE
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l17 and cytokine receptor
L17 NOT FOUND
The L-number entered could not be found.  To see the definition
of L-numbers, enter DISPLAY HISTORY at an arrow prompt (=>).

=> s l7 and cytokine receptor
L8            13 L7 AND CYTOKINE RECEPTOR

=> dup remove l8
PROCESSING COMPLETED FOR L8
L9            13 DUP REMOVE L8 (0 DUPLICATES REMOVED)

=> d l9 1-3 cbib abs

L9   ANSWER 1 OF 13  CAPLUS  COPYRIGHT 2002 ACS
2002:90109   Document No. 136:117385  Multi-specific reagent for selective
     stimulation of cell surface receptors.  Jung, Gundram (Germany).  PCT Int.
     Appl. WO 2002008291 A2 20020131, 28 pp.  DESIGNATED STATES: W:  AE, AG,
     AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ,
     DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN,
     IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK,
     MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
     TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU,
     TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR,
     GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR.
     (German).  CODEN: PIXXD2.  APPLICATION: WO 2001-EP8147 20010714.
     PRIORITY: DE 2000-10034607 20000720.
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Germany. DRUGS & AGING (1 SEP 2001) Vol. 18, No. 9, pp. 639-664. Publisher: ADIS INTERNATIONAL LTD. 41 CENTORIAN DR, PRIVATE BAG 65901, MAIRANGI BAY, AUCKLAND 10, NEW ZEALAND. ISSN: 1170-229X. Pub. country: Germany. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The concept of immunotherapy of cancer is more than a century old, but only recently have molecularly defined therapeutic approaches been developed. In this review, we focus on the most promising approach, active therapeutic vaccination.

The identification of tumour antigens can now be accelerated by methods allowing the amplification of gene products selectively or preferentially transcribed in the tumour. However, determining the potential immunogenicity of such gene products remains a demanding task, since major histocompatibility complex (MHC) restriction of T cells implies that for any newly defined antigen, immunogenicity will have to be defined for any individual MHC haplotype. Tumour-derived peptides elated from MHC molecules of tumour tissue are also a promising source of antigen.

Tumour antigens are mostly of weak immunogenicity, because the vast majority are tumour-associated differentiation antigens already 'seen' by the patient's immune system. Effective therapeutic vaccination will thus require adjuvant support, possibly by new approaches to immunomodulation such as **bispecific** antibodies or antibody-**cytokine** fusion proteins. Tumour-specific antigens, which could be a more potent target for immunotherapy, mostly arise by point mutations and have the disadvantage of being not only tumour-specific, but also individual-specific. Therapeutic vaccination will probably focus on defined antigens offered as protein, peptide or nucleic acid. Irrespective of the form in which the antigen is applied, emphasis will be given to the activation of dendritic cells as professional antigen presenters. Dendritic cells may be loaded in vitro with antigen, or, alternatively, initiation of an immune response may be approached in vivo by vaccination with RNA or DNA, given as such or packed into attenuated bacteria.

The importance of activation of T helper cells has only recently been taken into account in cancer vaccination. Activation of cytotoxic T cells is facilitated by the provision of T helper cell-derived **cytokines** . T helper cell-dependent recruitment of elements of non-adaptive defence, such as leucocytes, natural killer cells and monocytes, is of particular importance when the tumour has lost MHC class I expression.

Barriers to successful therapeutic vaccination include: (i) the escape mechanisms developed by tumour cells in response to immune attack; (ii) tolerance or anergy of the evoked immune response; (iii) the theoretical possibility of provoking an autoimmune reaction by vaccination against tumour-associated antigens; and (iv) the advanced age of many patients, implying reduced responsiveness of the senescent immune system.

L6 ANSWER 3 OF 3 MEDLINE DUPLICATE 2

1998112835 Document Number: 98112835. PubMed ID: 9446596. Recombinant human **single chain Fv** antibodies recognizing human interleukin-6. Specific targeting of **cytokine**-secreting cells. Krebs B; Griffin H; Winter G; Rose-John S. (Department of Medicine, Section of Pathophysiology, Johannes Gutenberg-University of Mainz, Obere Zahlbacher Strasse 63, D-55101 Mainz, Germany.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Jan 30) 273 (5) 2858-65. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB A human antibody library was displayed on the surface of filamentous bacteriophage and screened for binding to human interleukin-6 (IL-6). Two antibody-bearing phages were selected that bound IL-6. The complementary-determining region 3 loops of the variable heavy chains of these two antibodies differed in length and sequence and recognized two distinct epitopes. One of the **single chain Fv** fragments isolated (H1) was found to bind human (but not murine) IL-6 with an affinity comparable to that of the human IL-6 receptor. H1 also recognized newly synthesized human IL-6 intracellularly, as shown by

AB The invention relates to a multi-specific reagent comprising at least one first binding site for a cell surface receptor which requires a multimer ligand bond for the stimulation thereof. The reagent comprises a second binding site for a target antigen which is expressed on the same cell as the cell surface receptor. The reagent is preferably a **bispecific** antibody which can induce apoptosis of tumor cells or T cells or activation of antigen-presenting cells.

L9 ANSWER 2 OF 13 CAPLUS COPYRIGHT 2002 ACS
2002:814662 Document No. 137:293574 **Bispecific** antibodies that bind TRAIL-R1 and TRAIL-R2 for treatment of cancer and viral infection. Lynch, David H. (USA). U.S. Pat. Appl. Publ. US 2002155109 A1 20021024, 13 pp. (English). CODEN: USXXCO. APPLICATION: US 2001-839632 20010420.
AB **Bispecific** antibodies that bind TRAIL receptor 1 and TRAIL receptor 2 are provided. **Bispecific** antibodies that induce apoptosis of tumor cells and virally infected cells are employed in treating cancer and viral infections. The cancer cell can be leukemia, lymphoma, melanoma, breast carcinoma, colon carcinoma, and colorectal cancer, and the virus is human immunodeficiency virus.

L9 ANSWER 3 OF 13 CAPLUS COPYRIGHT 2002 ACS
2001:868530 Document No. 136:19113 **Bispecific** immunoglobulin-like antigen binding proteins and method of production. Zhu, Zhenping (Imclone Systems Incorporated, USA). PCT Int. Appl. WO 2001090192 A2 20011129, 64 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US16924 20010524. PRIORITY: US 2000-PV206749 20000524.
AB The present invention is directed to **bispecific** antigen-binding protein. These **bispecific** antigen-binding proteins are optimized in their avidity for antigen(s) but maintain their ability to function as a natural antibody, including the ability to activate complement mediated cytotoxicity and antibody dependent cellular toxicity. Natural IgG Igs are monospecific and bivalent, having two binding domains which are specific for the same epitope. By contrast, an IgG type antigen-binding protein of the invention is **bispecific** and bivalent, having a binding domain on each light chain for one epitope and a binding domain on each heavy chain specific for a second epitope. The design of the present antigen-binding proteins provides for efficient prodn. such that substantially all of the antigen-binding proteins produced are assembled in the desired configuration.

=> s bispecific and KDR
L10 16 BISPECIFIC AND KDR

=> dup remove l10
PROCESSING COMPLETED FOR L10
L11 6 DUP REMOVE L10 (10 DUPLICATES REMOVED)

=> d l11 1-6 cbib abs

L11 ANSWER 1 OF 6 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
2002:557780 Document No.: PREV200200557780. Fab-scFv fusion protein: An efficient approach to production of **bispecific** antibody fragments. Lu, Dan; Jimenez, Xenia; Zhang, Haifan; Bohlen, Peter; Witte, Larry; Zhu, Zhenping (1). (1) Department of Antibody Technology, ImClone Systems Incorporated, 180 Varick Street, New York, NY, 10014:

Zhenping@imclone.com USA. Journal of Immunological Methods, (15 September, 2002) Vol. 267, No. 2, pp. 213-226. http://www.elsevier.com/locate/jim. print. ISSN: 0022-1759. Language: English.

AB The clinical development of **bispecific** antibodies (BsAb) as therapeutics has been hampered by the difficulty in preparing the materials in sufficient quantity and quality by traditional methods. Here, we describe an efficient approach for the production of a novel **bispecific** antibody fragment by genetically fusing a single-chain Fv (scFv) to the C-terminus of either the light chain or the heavy chain of a Fab fragment of different antigen-binding specificity. The **bispecific** Fab-scFv fragments were expressed in a single Escherichia coli host and purified to homogeneity by a one-step affinity chromatography. Two different versions of the **bispecific** Fab-scFv fragments were constructed using two antibodies directed against the two tyrosine kinase receptors of vascular endothelial growth factor. These **bispecific** antibody fragments not only retained the antigen-binding capacity of each of the parent antibodies, but also are capable of binding to both targets simultaneously as demonstrated by a cross-linking ELISA. Further, the **bispecific** antibodies were comparable to their parent antibodies in their potency in blocking ligand binding to the receptors and in inhibiting ligand-induced biological activities. This design for BsAb fragments should be applicable to any pair of antigen specificities.

L11 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2002 ACS

2001:868530 Document No. 136:19113 **Bispecific** immunoglobulin-like antigen binding proteins and method of production. Zhu, Zhenping (Imclone Systems Incorporated, USA). PCT Int. Appl. WO 2001090192 A2 20011129, 64 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US16924 20010524. PRIORITY: US 2000-PV206749 20000524.

AB The present invention is directed to **bispecific** antigen-binding protein. These **bispecific** antigen-binding proteins are optimized in their avidity for antigen(s) but maintain their ability to function as a natural antibody, including the ability to activate complement mediated cytotoxicity and antibody dependent cellular toxicity. Natural IgG Igs are monospecific and bivalent, having two binding domains which are specific for the same epitope. By contrast, an IgG type antigen-binding protein of the invention is **bispecific** and bivalent, having a binding domain on each light chain for one epitope and a binding domain on each heavy chain specific for a second epitope. The design of the present antigen-binding proteins provides for efficient prodn. such that substantially all of the antigen-binding proteins produced are assembled in the desired configuration.

L11 ANSWER 3 OF 6 MEDLINE

2001538782 Document Number: 21469635. PubMed ID: 11585724. Complete inhibition of vascular endothelial growth factor (VEGF) activities with a bifunctional diabody directed against both VEGF kinase receptors, fms-like tyrosine kinase receptor and kinase insert domain-containing receptor. Lu D; Jimenez X; Zhang H; Wu Y; Bohlen P; Witte L; Zhu Z. (Department of Molecular and Cell Biology, ImClone Systems Inc., New York, New York 10014, USA.) CANCER RESEARCH, (2001 Oct 1) 61 (19) 7002-8. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Vascular endothelial growth factor (VEGF) binds to and mediates its activity mainly through two tyrosine kinase receptors, VEGF receptor 1 [or fms-like tyrosine kinase receptor (Flt-1)] and VEGF receptor 2 [or kinase

insert domain-containing receptor (**KDR**)]. Numerous studies have shown that overexpression of VEGF and its receptor plays an important role in tumor-associated angiogenesis and hence in both tumor growth and metastasis. We demonstrated previously that antagonistic antibodies to **KDR** specifically inhibited VEGF-stimulated receptor activation, cell migration, and endothelial cell mitogenesis. Here we constructed a recombinant bifunctional diabody that is capable of blocking both Flt-1 and **KDR** from binding to their ligands, including VEGF and placenta growth factor (PlGF). The diabody was expressed in Escherichia coli and purified by single-step affinity chromatography. The diabody retained the capacity to bind both **KDR** and Flt-1 and effectively blocked interaction between **KDR** and VEGF, Flt-1 and VEGF, and Flt-1 and PlGF. Furthermore, the diabody is a stronger inhibitor than its parent antibodies to VEGF-stimulated mitogenesis of human endothelial cells, as well as both VEGF- and PlGF-induced migration of human leukemia cells. Taken together, our results suggest that dual receptor blockade with the bifunctional diabody may prove to be a more efficient approach in inhibiting VEGF-stimulated angiogenesis.

L11 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2002 ACS

2000:260057 Document No. 132:298824 Flt4 (VEGFR-3) as a target for tumor imaging and anti-tumor therapy. Alitalo, Kari; Kaipainen, Arja; Valltola, Reija; Jussila, Lotta (Ludwig Institute for Cancer Research, USA; Helsinki University Licensing Ltd. Oy). PCT Int. Appl. WO 2000021560 A1 20000420, 148 pp. DESIGNATED STATES: W: AU, CA, CN, JP, NO, NZ; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US23525 19991008. PRIORITY: US 1998-169079 19981009.

AB The present invention provides purified Flt4 receptor tyrosine kinase polypeptides and fragments thereof, polynucleotides encoding such polypeptides, antibodies that specifically bind such polypeptides, and uses therefor.

L11 ANSWER 5 OF 6 MEDLINE DUPLICATE 2

2000295268 Document Number: 20295268. PubMed ID: 10835110. An efficient route to the production of an IgG-like **bispecific** antibody. Zuo Z; Jimenez X; Witte L; Zhu Z. (Department of Molecular and Cell Biology, ImClone Systems Incorporated, 180 Varick Street, New York, NY 10014, USA.) PROTEIN ENGINEERING, (2000 May) 13 (5) 361-7. Journal code: 8801484. ISSN: 0269-2139. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Production of IgG-form **bispecific** antibody (BsAb-IgG) by co-expressing two antibodies in transfected cells is often inefficient owing to the unwanted pairing between the component heavy and light chains. We have developed an efficient method for the production of a novel IgG-like BsAb by using the natural dimerization mechanism between IgG heavy and light chains. Two single-chain Fv (scFv) of different specificity are fused to the constant domain of human kappa chain (C(L)) and the first constant domain of human heavy chain (C(H1)), to form two polypeptides, (scFv)(1)-C(L) and (scFv)(2)-C(H1)-C(H2)-C(H3), respectively. Co-expression of the two polypeptides in mammalian cells results in the formation of a covalently linked IgG-like hetero-tetramer, Bs(scFv)(4)-IgG, with dual specificity. Our approach yields a homogeneous **bispecific** IgG-like antibody product with each molecule containing four antigen binding sites, two for each of its target antigens. A Bs(scFv)(4)-IgG was prepared using two scFv antibodies each directed against a different epitope of a vascular endothelial growth factor receptor, the kinase insert domain-containing receptor (**KDR**). The Bs(scFv)(4)-IgG is capable of simultaneously binding to the two epitopes on the receptor. Further, the Bs(scFv)(4)-IgG also retains the antigen-binding efficacy and biological activity of its component antibodies.

L11 ANSWER 6 OF 6 MEDLINE DUPLICATE 3

2000062952 Document Number: 20062952. PubMed ID: 10594363. Acquired antagonistic activity of a **bispecific** diabody directed against two different epitopes on vascular endothelial growth factor receptor 2. Lu D; Kotanides H; Jimenez X; Zhou Q; Persaud K; Bohlen P; Witte L; Zhu Z. (Department of Molecular and Cell Biology, ImClone Systems, 180 Varick Street, New York, NY 10014, USA.) JOURNAL OF IMMUNOLOGICAL METHODS, (1999 Nov 19) 230 (1-2) 159-71. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB **Bispecific** antibody (BsAb) technology has been successfully used as a means to construct novel antibody (Ab) molecules with increased avidity for binding, by combining two Ab or their fragments directed against different epitopes within the same antigen. Using two single chain antibodies (scFv) isolated from a phage display library, we have constructed a **bispecific** diabody directed against two different epitopes on the extracellular domain (ECD) of human vascular endothelial growth factor receptor 2 (VEGFR2), the kinase-insert domain-containing receptor (**KDR**). Neither of the parent scFv blocks **KDR** /VEGF interactions or inhibits VEGF-induced receptor activation. The diabody binds to **KDR** with an affinity that is 1.5- to 3-fold higher than its parent scFv, mainly due to a much slower dissociation rate (k(off)), which is approximately 17- to 26-fold slower than that of the individual scFv. In addition, the diabody binds simultaneously to, and thus cross-links, the two epitopes on the receptor(s). It is rather unexpected that the diabody effectively blocked **KDR**/VEGF interactions, and inhibited both VEGF-induced activation of the receptor and mitogenesis of human endothelial cells. Taken together, our results suggest that the diabody is most likely to exert its effect through steric hindrance and/or causing major conformational changes of the receptor. This is the first report on the construction of a **bispecific** diabody with acquired novel antagonistic activity.

=> s bispecific and Flk-1
L12 1 BISPECIFIC AND FLK-1

=> d l12 cbib abs

L12 ANSWER 1 OF 1 SCISEARCH COPYRIGHT 2002 ISI (R)

1999:962836 The Genuine Article (R) Number: 263WR. Acquired antagonistic activity of a **bispecific** diabody directed against two different epitopes on vascular endothelial growth factor receptor 2. Lu D; Kotanides H; Jimenez X; Zhou Q W; Persaud K; Bohlen P; Witte L; Zhu Z P (Reprint). IMCLONE SYST, DEPT MOL & CELL BIOL, 180 VARICK ST, NEW YORK, NY 10014 (Reprint); IMCLONE SYST, DEPT MOL & CELL BIOL, NEW YORK, NY 10014; IMCLONE SYST, DEPT PROT CHEM, NEW YORK, NY 10014; IMCLONE SYST, DEPT RES, NEW YORK, NY 10014. JOURNAL OF IMMUNOLOGICAL METHODS (19 NOV 1999) Vol. 230, No. 1-2, pp. 159-171. Publisher: ELSEVIER SCIENCE BV. PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 0022-1759. Pub. country: USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Bispecific** antibody (BsAb) technology has been successfully used as a means to construct novel antibody (Ab) molecules with increased avidity for binding, by combining two Ab or their fragments directed against different epitopes within the same antigen. Using two single chain antibodies (scFv) isolated from a phage display library, we have constructed a **bispecific** diabody directed against two different epitopes on the extracellular domain (ECD) of human vascular endothelial growth factor receptor 2 (VEGFR2), the kinase-insert domain-containing receptor (KDR). Neither of the parent scFv blocks KDR/VEGF interactions or inhibits VEGF-induced receptor activation. The diabody binds to KDR with an affinity that is 1.5- to 3-fold higher than its parent scFv, mainly due to a much slower dissociation rate (k(off)), which is approximately 17- to 26-fold slower than that of the individual scFv. In addition, the diabody

binds simultaneously to, and thus cross-links, the two epitopes on the receptor(s). It is rather unexpected that the diabody effectively blocked KDR/VEGF interactions, and inhibited both VEGF-induced activation of the receptor and mitogenesis of human endothelial cells. Taken together, our results suggest that the diabody is most likely to exert its effect through steric hindrance and/or causing major conformational changes of the receptor. This is the first report on the construction of a **bispecific** diabody with acquired novel antagonistic activity. (C) 1999 Elsevier Science B.V. All rights reserved.

=> s bispecific and Flt-1
L13 8 BISPECIFIC AND FLT-1

=> dup remove l13
PROCESSING COMPLETED FOR L13
L14 6 DUP REMOVE L13 (2 DUPLICATES REMOVED)

=> d l14 1-6 cbib abs

L14 ANSWER 1 OF 6 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
2002:557780 Document No.: PREV200200557780. Fab-scFv fusion protein: An efficient approach to production of **bispecific** antibody fragments. Lu, Dan; Jimenez, Xenia; Zhang, Haifan; Bohlen, Peter; Witte, Larry; Zhu, Zhenping (1). (1) Department of Antibody Technology, ImClone Systems Incorporated, 180 Varick Street, New York, NY, 10014: Zhenping@imclone.com USA. Journal of Immunological Methods, (15 September, 2002) Vol. 267, No. 2, pp. 213-226. http://www.elsevier.com/locate/jim. print. ISSN: 0022-1759. Language: English.
AB The clinical development of **bispecific** antibodies (BsAb) as therapeutics has been hampered by the difficulty in preparing the materials in sufficient quantity and quality by traditional methods. Here, we describe an efficient approach for the production of a novel **bispecific** antibody fragment by genetically fusing a single-chain Fv (scFv) to the C-terminus of either the light chain or the heavy chain of a Fab fragment of different antigen-binding specificity. The **bispecific** Fab-scFv fragments were expressed in a single Escherichia coli host and purified to homogeneity by a one-step affinity chromatography. Two different versions of the **bispecific** Fab-scFv fragments were constructed using two antibodies directed against the two tyrosine kinase receptors of vascular endothelial growth factor. These **bispecific** antibody fragments not only retained the antigen-binding capacity of each of the parent antibodies, but also are capable of binding to both targets simultaneously as demonstrated by a cross-linking ELISA. Further, the **bispecific** antibodies were comparable to their parent antibodies in their potency in blocking ligand binding to the receptors and in inhibiting ligand-induced biological activities. This design for BsAb fragments should be applicable to any pair of antigen specificities.

L14 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2002 ACS
2002:674715 Document No. 137:210588 Intraperitoneal **bispecific** antibody (HEA125XOKT3) therapy inhibits malignant ascites production in advanced ovarian carcinoma. Marme, Alexander; Strauss, Gudrun; Bastert, Gunther; Grischke, Eva-Maria; Moldenhauer, Gerhard (Department of Obstetrics and Gynecology, University of Heidelberg, Heidelberg, Germany). International Journal of Cancer, 101(2), 183-189 (English) 2002. CODEN: IJCNAW. ISSN: 0020-7136. Publisher: Wiley-Liss, Inc..
AB **Bispecific** antibody HEA125xOKT3 was shown to redirect T lymphocytes toward carcinoma cells and to induce tumor cell lysis in vitro. Preclin. studies have demonstrated that tumor-assocd. lymphocytes (TAL) derived from malignant ascites can be used as effector cells with a high efficacy and without prior stimulation. These data provided the

rationale for a clin. trial to investigate whether bsAb HEA125xOKT3 is also able to induce tumor cell lysis in vivo and can be used for local treatment of malignant ascites arising from ovarian carcinoma. Ten ovarian carcinoma patients presenting with malignant ascites resistant to chemotherapy were enrolled in the study. They received weekly i.p. injections of 1 mg bsAb dild. in 500 mL NaCl soln. to allow homogeneous antibody distribution within the peritoneal cavity. All patients responded clin. well to the therapy. Eight patients experienced a complete and 2 patients a partial redn. of ascites prodn. A decrease or stabilization of tumor marker CA125 was detected in the sera of 8 patients. Only WHO Grade I and II toxicity was obsd. including mild fever, chills and allergic eczema. Thus, i.p. application of bsAb HEA125xOKT3 appears to be an easy and cost effective palliative treatment for ovarian carcinoma with recurrent ascites that leads to a substantially increased quality of life for the patients. During therapy TNF-.alpha. concns. raised markedly in the ascites fluid whereas VEGF and sFLT-1 ascites levels declined. This indicates that not only T cell-mediated tumor cell lysis but also changes in vascular permeability due to downregulation of VEGF and its receptors might be responsible for the beneficial therapeutic effect.

L14 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2002 ACS

2001:868530 Document No. 136:19113 **Bispecific** immunoglobulin-like antigen binding proteins and method of production. Zhu, Zhenping (Imclone Systems Incorporated, USA). PCT Int. Appl. WO 2001090192 A2 20011129, 64 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US16924 20010524. PRIORITY: US 2000-PV206749 20000524.

AB The present invention is directed to **bispecific** antigen-binding protein. These **bispecific** antigen-binding proteins are optimized in their avidity for antigen(s) but maintain their ability to function as a natural antibody, including the ability to activate complement mediated cytotoxicity and antibody dependent cellular toxicity. Natural IgG Igs are monospecific and bivalent, having two binding domains which are specific for the same epitope. By contrast, an IgG type antigen-binding protein of the invention is **bispecific** and bivalent, having a binding domain on each light chain for one epitope and a binding domain on each heavy chain specific for a second epitope. The design of the present antigen-binding proteins provides for efficient prodn. such that substantially all of the antigen-binding proteins produced are assembled in the desired configuration.

L14 ANSWER 4 OF 6 MEDLINE

2001538782 Document Number: 21469635. PubMed ID: 11585724. Complete inhibition of vascular endothelial growth factor (VEGF) activities with a bifunctional diabody directed against both VEGF kinase receptors, fms-like tyrosine kinase receptor and kinase insert domain-containing receptor. Lu D; Jimenez X; Zhang H; Wu Y; Bohlen P; Witte L; Zhu Z. (Department of Molecular and Cell Biology, ImClone Systems Inc., New York, New York 10014, USA.) CANCER RESEARCH, (2001 Oct 1) 61 (19) 7002-8. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Vascular endothelial growth factor (VEGF) binds to and mediates its activity mainly through two tyrosine kinase receptors, VEGF receptor 1 [or fms-like tyrosine kinase receptor (**Flt-1**)] and VEGF receptor 2 [or kinase insert domain-containing receptor (KDR)]. Numerous studies have shown that overexpression of VEGF and its receptor plays an important role in tumor-associated angiogenesis and hence in both tumor

growth and metastasis. We demonstrated previously that antagonistic antibodies to KDR specifically inhibited VEGF-stimulated receptor activation, cell migration, and endothelial cell mitogenesis. Here we constructed a recombinant bifunctional diabody that is capable of blocking both **Flt-1** and KDR from binding to their ligands, including VEGF and placenta growth factor (PlGF). The diabody was expressed in Escherichia coli and purified by single-step affinity chromatography. The diabody retained the capacity to bind both KDR and **Flt-1** and effectively blocked interaction between KDR and VEGF, **Flt-1** and VEGF, and **Flt-1** and PlGF. Furthermore, the diabody is a stronger inhibitor than its parent antibodies to VEGF-stimulated mitogenesis of human endothelial cells, as well as both VEGF- and PlGF-induced migration of human leukemia cells. Taken together, our results suggest that dual receptor blockade with the bifunctional diabody may prove to be a more efficient approach in inhibiting VEGF-stimulated angiogenesis.

L14 ANSWER 5 OF 6 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2002:130441 Document No.: PREV200200130441. Combined transductional and transcriptional targeting improves the specificity of transgene expression in vivo. Reynolds, Paul N. (1); Nicklin, Stuart A.; Kaliberova, Lioudmila; Boatman, Brian G.; Grizzle, William E.; Balyasnikova, Irina V.; Baker, Andrew H.; Danilov, Sergei M.; Curiel, David T.. (1) Division of Human Gene Therapy, Departments of Medicine, Surgery and Pathology, and Gene Therapy Center, University of Alabama at Birmingham, Birmingham, AL: Paul.Reynolds@ccc.uab.edu USA. Nature Biotechnology, (September, 2001) Vol. 19, No. 9, pp. 838-842. http://www.nature.com/nbt/. print. ISSN: 1087-0156. Language: English.

AB The promise of gene therapy for health care will not be realized until gene delivery systems are capable of achieving efficient, cell-specific gene delivery in vivo. Here we describe an adenoviral system for achieving cell-specific transgene expression in pulmonary endothelium. The combination of transductional targeting to a pulmonary endothelial marker (angiotensin-converting enzyme, ACE) and an endothelial-specific promoter (for vascular endothelial growth factor receptor type 1, **flt-1**) resulted in a synergistic, 300,000-fold improvement in the selectivity of transgene expression for lung versus the usual site of vector sequestration, the liver. This combined approach should be useful for the design of other gene delivery systems.

L14 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2002 ACS
2000:260057 Document No. 132:298824 Flt4 (VEGFR-3) as a target for tumor imaging and anti-tumor therapy. Alitalo, Kari; Kaipainen, Arja; Valltola, Reija; Jussila, Lotta (Ludwig Institute for Cancer Research, USA; Helsinki University Licensing Ltd. Oy). PCT Int. Appl. WO 2000021560 A1 20000420, 148 pp. DESIGNATED STATES: W: AU, CA, CN, JP, NO, NZ; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US23525 19991008. PRIORITY: US 1998-169079 19981009.

AB The present invention provides purified Flt4 receptor tyrosine kinase polypeptides and fragments thereof, polynucleotides encoding such polypeptides, antibodies that specifically bind such polypeptides, and uses therefor.

=> s bispecific and FGF receptor
L15 0 BISPECIFIC AND FGF RECEPTOR

=> s bispecific and PDGF-R
L16 0 BISPECIFIC AND PDGF-R

=> s bispecific and EGF receptor
L17 70 BISPECIFIC AND EGF RECEPTOR

=> dup remove l17
PROCESSING COMPLETED FOR L17
L18 27 DUP REMOVE L17 (43 DUPLICATES REMOVED)

=> s l18 and single chain
L19 8 L18 AND SINGLE CHAIN

=> dup remove l19
PROCESSING COMPLETED FOR L19
L20 8 DUP REMOVE L19 (0 DUPLICATES REMOVED)

=> d l20 1-8 cbib abs

L20 ANSWER 1 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2002:419008 Document No.: PREV200200419008. Production and evaluation of **bispecific single-chain** Fv molecules that target HER2/neu and HER3. Horak, Eva M. (1); Shahied, Lillian S.; Shaller, Calvin C.; Tesfaye, Abohawariat; Simmons, Heidi H.; Alpaugh, R. Katherine; Greer, Nathaniel B.; Heitner, Tara; Garrison, Jennifer L.; Marks, James D.; Weiner, Louis M.; Adams, Gregory P.. (1) Fox Chase Cancer Center, Philadelphia, PA USA. Proceedings of the American Association for Cancer Research Annual Meeting, (March, 2002) Vol. 43, pp. 971. print. Meeting Info.: 93rd Annual Meeting of the American Association for Cancer Research San Francisco, California, USA April 06-10, 2002 ISSN: 0197-016X. Language: English.

L20 ANSWER 2 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2002:1515 Document No.: PREV200200001515. Engineering **bispecific single-chain** Fv molecules to alter signaling of the **EGF receptor** family. Horak, Eva M. (1); Heitner, Tara; Garrison, Jennifer L.; Simmons, Heidi H.; Alpaugh, R. Katherine; Amoroso, Anne R.; Marks, James D.; Weiner, Louis M.; Adams, Gregory P.. (1) Fox Chase Cancer Center, Philadelphia, PA USA. Proceedings of the American Association for Cancer Research Annual Meeting, (March, 2001) Vol. 42, pp. 774. print. Meeting Info.: 92nd Annual Meeting of the American Association for Cancer Research New Orleans, LA, USA March 24-28, 2001 ISSN: 0197-016X. Language: English.

L20 ANSWER 3 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:157342 Document No.: PREV200100157342. Selection of cell binding and internalizing epidermal growth factor receptor antibodies from a phage display library. Heitner, Tara; Moor, Anne; Garrison, Jennifer L.; Marks, Cara; Hasan, Tayyaba; Marks, James D. (1). (1) Departments of Anesthesia and Pharmaceutical Chemistry, University of California, San Francisco, San Francisco General Hospital, 1001 Potrero Avenue, Room 3C-38, San Francisco, CA, 94110: marksj@anesthesia.ucsf.edu USA. Journal of Immunological Methods, (1 February, 2001) Vol. 248, No. 1-2, pp. 17-30. print. ISSN: 0022-1759. Language: English. Summary Language: English.
AB The first step in developing a targeted cancer therapeutic is generating a ligand that binds to a receptor which is either tumor specific or sufficiently overexpressed in tumors to provide targeting specificity. For this work, we generated human monoclonal antibodies to the **EGF receptor** (EGFR), an antigen overexpressed on many solid tumors. **Single chain** Fv (scFv) antibody fragments were directly selected by panning a phage display library on tumor cells (A431) overexpressing EGFR or Chinese hamster ovary cells (CHO/EGFR cells) transfected with the EGFR gene and recovering endocytosed phage from within the cell. Three unique scFvs were isolated, two from selections on A431 cells and two from selections on CHO/EGFR cells. All three scFv bound native receptor as expressed on a panel of tumor cells and did not bind EGFR negative cells. Phage antibodies and multivalent immunoliposomes constructed from scFv were endocytosed by EGFR expressing cells as shown

by confocal microscopy. Native scFv primarily stained the cell surface, with less staining intracellularly. The results demonstrate how phage antibodies binding native cell surface receptors can be directly selected on overexpressing cell lines or transfected cells. Use of a transfected cell line allows selection of antibodies to native receptors without the need for protein expression and purification, significantly speeding the generation of targeting antibodies to genomic sequences. Depending upon the format used, the antibodies can be used to deliver molecules to the cell surface or intracellularly.

L20 ANSWER 4 OF 8 SCISEARCH COPYRIGHT 2002 ISI (R)
2000:882198 The Genuine Article (R) Number: 374LX. Construction and characterization of **bispecific** costimulatory molecules containing a minimized CD86 (B7-2) domain and **single-chain** antibody fragments for tumor targeting. Rohrbach F; Gerstmayer B; Biburger M; Wels W (Reprint). GEORG SPEYER HAUS, CHEMOTHERAPEUT FORSCHUNGSINST, PAUL EHRLICH STR 42-44, D-60596 FRANKFURT, GERMANY (Reprint); GEORG SPEYER HAUS, CHEMOTHERAPEUT FORSCHUNGSINST, D-60596 FRANKFURT, GERMANY; MEMOREC STOFFEL GMBH, D-50829 COLOGNE, GERMANY . CLINICAL CANCER RESEARCH (NOV 2000) Vol. 6, No. 11, pp. 4314-4322. Publisher: AMER ASSOC CANCER RESEARCH. PO BOX 11806, BIRMINGHAM, AL 35202. ISSN: 1078-0432. Pub. country: GERMANY. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Efficient T-cell activation requires two signals. The first signal, which confers specificity, is provided hy interaction of the T-cell receptor with peptides presented by MHC molecules, One of the second costimulatory signals is induced by binding of B7 proteins on the surface of antigen-presenting cells to CD28 on the T-cell surface. Expression of B7 molecules on tumor cells can result in the activation of tumor specific T lymphocytes and induce protective antitumor immunity. However, at present such gene-therapeutic approaches are limited by the inability to selectively target B7 gene expression to cancer cells, As an alternative approach we exploited recombinant antibody fragments to localize a costimulatory B7 molecule to the surface of tumor cells. We constructed chimeric proteins that contain in a single polypeptide chain a portion of human B7-2 (CD86) genetically fused to **single-chain** (sc) Fv antibody domains specific for the tumor-associated antigens epidermal growth factor receptor and the closely related ErbB2 receptor tyrosine kinase, A small recombinant fragment of human CD86 was characterized that corresponds to amino acid residues 1-111 (CD86(111)) of the mature protein. CD86(111) produced in the yeast Pichia pastoris and CD86(111) expressed in bacteria was functionally active and displayed specific binding to B7 counter receptors, Bacterially expressed CD86(111)-scFv fusion proteins specifically localized to the respective target antigens on the surface of tumor cells and markedly enhanced the proliferation of primary T cells when bound to immobilized tumor antigen.

L20 ANSWER 5 OF 8 MEDLINE
1998149664 Document Number: 98149664. PubMed ID: 9490020. The first constant domain (C(H)1 and C(L)) of an antibody used as heterodimerization domain for **bispecific** miniantibodies. Muller K M; Arndt K M; Strittmatter W; Pluckthun A. (Biochemisches Institut, Universitat Zurich, Switzerland.) FEBS LETTERS, (1998 Jan 30) 422 (2) 259-64. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.
AB **Bispecific** miniantibodies were constructed by genetically fusing the C(H)1 domain of an IgG1 to the C-terminus of a **single-chain** Fv fragment (scFv-425), specific for the **EGF receptor**, and fusing the C(L) domain of a kappa light chain to the C-terminus of a scFv specific for CD2 (scFv-M1). An efficient dicistronic gene arrangement for functional expression in Escherichia coli was constructed. Immunoblots demonstrated correct domain assembly and the formation of the natural C(H)1-C(L) disulfide bridge. Gel filtration confirmed the correct size, sandwich ELISAs demonstrated

bispecific functionality, and SPR biosensor measurements determined binding to EGF-R in comparison to bivalent constructs. **Bispecific** anti-EGF-R/anti-CD2 miniantibodies are candidates for the immunotherapy of cancer.

L20 ANSWER 6 OF 8 MEDLINE
1998374022 Document Number: 98374022. PubMed ID: 9710248. A dimeric **bispecific** miniantibody combines two specificities with avidity. Muller K M; Arndt K M; Pluckthun A. (Biochemisches Institut, Universitat Zurich, Switzerland.) FEBS LETTERS, (1998 Jul 31) 432 (1-2) 45-9. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB **Bispecific** antibodies extend the capabilities of nature and might be applied in immunotherapy and biotechnology. By fusing the gene of a **single-chain** Fv (scFv) fragment to a helical dimerization domain, followed by a second scFv fragment of different specificity, we were able to express a functional protein in E. coli, which is **bispecific** and has two valencies for each specificity. The dimeric **bispecific** (DiBi) miniantibody preserves the natural avidity of antibodies in a very small-sized molecule of only 120 kDa. The generality of the principle was shown with a scFv fragment binding the **EGF-receptor** (named scFv 425) in three combinations with scFv fragments either directed against CD2 (ACID2.M1), phosphorylcholine (McPC603) or fluorescein (FITC-E2). Binding was analyzed by sandwich surface plasmon resonance biosensor (BIAcore) measurements.

L20 ANSWER 7 OF 8 CAPLUS COPYRIGHT 2002 ACS
1996:654026 Document No. 125:292469 Targeted inhibition of tumor cell growth by a **bispecific single-chain** toxin containing an antibody domain and TGF.alpha.. Schmidt, M.; Wels, W. (Tumour Biology Center, Institute Experimental Cancer Research, Freiburg/Br., D-79011, Germany). British Journal of Cancer, 74(6), 853-862 (English) 1996. CODEN: BJCAAI. ISSN: 0007-0920. Publisher: Stockton.

AB The construction and functional characterization of a novel **bispecific** recombinant toxin, scFv(FRP5)-TGF.alpha.-ETA (scFv = epidermal growth factor receptor (EGFR)-specific **single-chain** Fv domains and ETA = Pseudomonas exotoxin A) were reported. The fusion protein consists of the antigen-binding domain of the ErbB-2-specific monoclonal antibody (MAb), FRP5, and the natural EGFR ligand TGF.alpha. inserted at different positions in truncated Pseudomonas exotoxin A. ScFv(FRP5)-TGF.alpha.-ETA protein displayed binding to EGFR and ErbB-2, thereby inducing activation of the receptors, which was dependent on the cellular context and the level of EGFR and ErbB-2 expression. The **bispecific** mol. was cytotoxic in vitro for tumor cells expressing various levels of the target receptors. In vivo scFv(FRP5)-TGF.alpha.-ETA potently inhibited the growth of established A431 tumor xenografts in nude mice.

L20 ANSWER 8 OF 8 MEDLINE
96184158 Document Number: 96184158. PubMed ID: 8621240. A bivalent **single-chain** antibody-toxin specific for ErbB-2 and the **EGF receptor**. Schmidt M; Hynes N E; Groner B; Wels W. (Institute for Experimental Cancer Research, Tumor Biology Center, Freiburg, Germany.) INTERNATIONAL JOURNAL OF CANCER, (1996 Feb 8) 65 (4) 538-46. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB ErbB-2 and **EGF receptors** are often co-expressed in human tumors and have been shown to synergize in the transformation of cells in experimental model systems. Transactivation of ErbB-2 can occur via ligand-induced heterodimerization with **EGF receptor** or other members of the ErbB family of receptor tyrosine kinases. We have previously described the potent anti-tumoral activity of the monospecific

single-chain antibody-toxins scFv(FRP5)-ETA and scFv(225)-ETA binding to, respectively, ErbB-2 and the **EGF receptor**. Here we report the construction and functional characterization of a novel bivalent, **bispecific single -chain** antibody-toxin, scFv2(FRP5/225)-ETA. The fusion protein consists of 2 scFv domains specific for ErbB-2 and the **EGF receptor** linked to a modified Pseudomonas exotoxin A. ScFv2(FRP5/225)-ETA displayed in vitro cell killing activity on tumor cells overexpressing either ErbB-2 or the **EGF receptor** similar to that of the monospecific toxins. It was more potent in vitro and in vivo in inhibiting the growth of tumor cells expressing both receptors. Treatment of A431 cells with scFv2(FRP5/225)-ETA led to an increase in **EGF receptor** and ErbB-2 phosphotyrosine content, most likely via the induction of receptor heterodimers. This may explain the enhanced toxicity of the **bispecific** antibody-toxin.

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L18 ANSWER 1 OF 27 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2002:419008 Document No.: PREV200200419008. Production and evaluation of **bispecific** single-chain Fv molecules that target HER2/neu and HER3. Horak, Eva M. (1); Shahied, Lillian S.; Shaller, Calvin C.; Tesfaye, Abohawariat; Simmons, Heidi H.; Alpaugh, R. Katherine; Greer, Nathaniel B.; Heitner, Tara; Garrison, Jennifer L.; Marks, James D.; Weiner, Louis M.; Adams, Gregory P.. (1) Fox Chase Cancer Center, Philadelphia, PA USA. Proceedings of the American Association for Cancer Research Annual Meeting, (March, 2002) Vol. 43, pp. 971. print. Meeting Info.: 93rd Annual Meeting of the American Association for Cancer Research San Francisco, California, USA April 06-10, 2002 ISSN: 0197-016X. Language: English.

L18 ANSWER 2 OF 27 CAPLUS COPYRIGHT 2002 ACS
2002:108621 Document No. 137:61806 Epidermal growth factor receptor and G250: Useful target antigens for antibody mediated cellular cytotoxicity against renal cell carcinoma?. Stadick, H.; Stockmeyer, B.; Kuhn, R.; Schrott, K. M.; Kalden, J. R.; Glennie, M. J.; Van De Winkel, J. G. J.; Gramatzki, M.; Valerius, T.; Elsasser, D. (Departments of Medicine III and Urology, University of Erlangen-Nurnberg, Erlangen, Germany). Journal of Urology (Hagerstown, MD, United States), 167(2, Pt. 1), 707-712 (English) 2002. CODEN: JOURAA. ISSN: 0022-5347. Publisher: Lippincott Williams & Wilkins.
AB Monoclonal antibodies are a novel treatment option for certain tumor patients. The authors evaluated the potential of antibody derivs. against epidermal growth factor receptor and G250, which are 2 candidate antigens on renal cell carcinoma, to recruit effector cells for killing renal cell carcinoma. As a measure of cytotoxicity, 51chromium release assays against renal cell carcinoma lines were performed using unsepd. blood or isolated cell populations as the source of effectors. Blood was obtained from healthy donors, or from patients receiving granulocyte-macrophage colony-stimulating factor or granulocyte colony-stimulating factor for enhancing effector cell function. Parental human IgG1 antibodies against epidermal growth factor receptor and G250 were compared with resp. chem. linked **bispecific** antibodies targeting IgA Fc receptor Fc.alpha.RI (CD89), a novel cytotoxic trigger mol. on polymorphonuclear cells and monocytes/macrophages, which were constructed by chem. crosslinking appropriate F(ab') fragments. Renal cell carcinoma lines were highly resistant to complement dependent lysis. With mononuclear effector cells high levels of renal cell carcinoma killing were obsd. with a humanized epidermal growth factor receptor directed monoclonal antibody, while the same antibody did not recruit granulocytes (polymorphonuclear cells) for antibody dependent cell mediated cytotoxicity. However, polymorphonuclear cells effectively lysed renal cell carcinoma with (Fc.alpha.RI .times. epidermal growth factor receptor) **bispecific**

antibody. Fc.alpha.RI mediated killing was enhanced when the blood of patients on granulocyte colony-stimulating factor or granulocyte-macrophage colony-stimulating factor therapy was analyzed. However, G250 mediated only low levels of killing with mononuclear cell but not with polymorphonuclear effector cells. Targeting epidermal growth factor receptor proved to recruit efficiently mononuclear or polymorphonuclear cell mediated killing mechanisms, while G250 directed antibody constructs were less effective. Particularly effective renal cell carcinoma killing was obsd. with combined (c.alpha.RI .times. epidermal growth factor receptor) **bispecific** antibody and granulocyte colony-stimulating factor or granulocyte-macrophage colony-stimulating factor.

L18 ANSWER 3 OF 27 MEDLINE DUPLICATE 1

2002366804 Document Number: 22106506. PubMed ID: 12111392. Human betacellulin structure modeled from other members of EGF family. Lopez-Torrejon Ines; Querol Enrique; Aviles Francesc X; Seno Masaharu; De Llorens Rafael; Oliva Baldomero. (Institut de Biotecnologia i de Biomedicina "Vicent Villar Palasi" and Departament de Bioquimica, Universitat Autonoma de Barcelona, 08193 Bellaterra, Barcelona, Spain E-mail: boliva@imim.es Phone: +34 93 5422933 Fax: +34 93 5422802.) J Mol Model (Online), (2002 Apr) 8 (4) 131-44. Journal code: 9806569. ISSN: 0948-5023. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB We have modeled betacellulin (BTC) to gain insight into the structural elements that can explain its properties. The epidermal growth factor (EGF) signal transduction pathway, a significant mediator of several cell functions, is based on four closely related tyrosine kinase receptors. The ErbB receptors are transmembrane glycoproteins and signal transduction is initiated by ligand binding that induces receptor homo- or heterodimerization to form a complex containing two molecules of ligand and two molecules of receptor. The EGF family of ligands can be divided into three groups based on their ability to bind and activate distinct ErbB receptor homo- and heterodimers. Each member of the group formed by BTC, heparin binding EGF (HB-EGF) and epiregulin (EP) can interact with both the **EGF receptor** (EGFR) and heregulin receptors (ErbB-3 and ErbB-4), and are hence called "**bispecific**" ligands. BTC and EP also present the distinctive feature that they activate all possible heterodimeric ErbB receptors. BTC has been modeled with the program MODELLER, using human EGF, human transforming growth factor alpha (hTGFalpha), human HB-EGF and human heregulin one alpha (hHRG-1alpha) as templates. The structure of the model as well as that of the templates were optimized and a simulation of 100 ps was run for all. The main structural properties of the model and the templates were compared and in conclusion the hBTC conformation was closely similar to that of hTGFalpha. Electronic supplementary material to this paper can be obtained by using the Springer LINK server located at http://dx.doi.org/10.1007/s00894-002-0072-2.

L18 ANSWER 4 OF 27 CAPLUS COPYRIGHT 2002 ACS

2002:79322 Document No. 137:62082 Anti-CD3/anti-epidermal growth factor receptor-**bispecific** antibody retargeting of lymphocytes against human neoplastic keratinocytes in an autologous organotypic culture model. Renard, Isabelle; Mezzanzanica, Delia; Canevari, Silvana; Ferrini, Silvano; Boniver, Jacques; Delvenne, Philippe; Jacobs, Nathalie (Department of Pathology, University of Liege, Liege, B-4000, Belg.). American Journal of Pathology, 160(1), 113-122 (English) 2002. CODEN: AJPAA4. ISSN: 0002-9440. Publisher: American Society for Investigative Pathology.

AB Local cellular immune defects have been described in several tumors including human papillomavirus (HPV)-assocd. cervical cancer. This observation suggests the potential therapeutic benefit of immune manipulations that restore cellular immunity. Here, the authors evaluated the ability of **bispecific** monoclonal antibodies (bimAbs) to

redirect T cells against keratinocytes transformed in vitro by HPV in an autologous three-dimensional culture model (organotypic cultures). The epidermal growth factor receptor (EGFR) was chosen as target for an anti-CD3/anti-EGFR bimAb because it is overexpressed in many malignant epithelial lesions and only weakly expressed in the basal layers of normal squamous epithelium. Interestingly, in organotypic cultures, the pattern of expression of EGFR was similar to that obsd. in vivo. The ability of T cells retargeted by CD3/EGFR bimAb to lyse HPV-transformed cell lines was confirmed in monolayer cultures. In autologous organotypic cultures, an increase in apoptotic HPV+ keratinocytes and a significant decrease in the thickness of HPV+ organotypic cultures were obsd. when activated lymphocytes and bimAbs were added to the cultures, whereas organotypic cultures of normal keratinocytes were not significantly affected. These data were similar to those obtained in the allogeneic model. These results suggest the potential usefulness of CD3-EGFR bimAb-retargeted lymphocytes in immunotherapeutic protocols for malignant epithelial lesions.

L18 ANSWER 5 OF 27 CAPLUS COPYRIGHT 2002 ACS

2001:12302 Document No. 134:91105 Humanized anti-ErbB2 antibody-maytansinoid conjugates and uses thereof in cancer therapy. Erickson, Sharon; Schwall, Ralph (Genentech, Inc., USA). PCT Int. Appl. WO 2001000244 A2 20010104, 92 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US17229 20000623. PRIORITY: US 1999-PV141316 19990625; US 2000-PV189844 20000316.

AB The application concerns methods of treatment using anti-ErbB receptor antibody-maytansinoid conjugates, and articles of manuf. suitable for use in such methods. In particular, the invention concerns ErbB receptor-directed cancer therapies, using anti-ErbB receptor antibody-maytansinoid conjugates. The present invention is based on the unexpected exptl. finding that HERCEPTIN-maytansinoid conjugates are highly effective in the treatment.of HER2 (ErbB2) overexpressing tumors that do not respond, or respond poorly, to HERCEPTIN.rho. therapy. In one aspect, the present invention concerns a method for the treatment of a tumor in a mammal, wherein the tumor is characterized by the overexpression of an ErbB receptor and does not respond or responds poorly to treatment with a monoclonal anti-ErbB antibody, comprising administering to the mammal a therapeutically effective amt. of a conjugate of the anti-ErbB antibody with a maytansinoid. The maytansinoid used in the conjugates of the present invention may be maytansine or, preferably, maytansinol or a maytansinol ester. The antibody and maytansinoid may be conjugated by a **bispecific** chem. linker,such as N-succinimidyl-4-(2-pyridylthio)propanoate (SPDP) or N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP). Thelinking group between the antibody and the maytansinoid may, for example, be a disulfide, thioether, acid labile,photolabile, peptidase labile, or esterase labile group. In another aspect, the invention concerns an article of manuf. comprising a container and a compn. contained therein, wherein the compn. comprises an anti-ErbB antibody-maytansinoid conjugate, and further comprising a package insert or label indicating that the compn. can be used to treat cancer characterized by overexpression of an ErbB receptor, preferably at a 2+ level or above.

L18 ANSWER 6 OF 27 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

2002:1515 Document No.: PREV200200001515. Engineering **bispecific** single-chain Fv molecules to alter signaling of the **EGF**

receptor family. Horak, Eva M. (1); Heitner, Tara; Garrison, Jennifer L.; Simmons, Heidi H.; Alpaugh, R. Katherine; Amoroso, Anne R.; Marks, James D.; Weiner, Louis M.; Adams, Gregory P.. (1) Fox Chase Cancer Center, Philadelphia, PA USA. Proceedings of the American Association for Cancer Research Annual Meeting, (March, 2001) Vol. 42, pp. 774. print. Meeting Info.: 92nd Annual Meeting of the American Association for Cancer Research New Orleans, LA, USA March 24-28, 2001 ISSN: 0197-016X. Language: English.

L18 ANSWER 7 OF 27 MEDLINE DUPLICATE 2
2001111735 Document Number: 20581961. PubMed ID: 11146449. Local immunotherapy of glioma patients with a combination of 2 **bispecific** antibody fragments and resting autologous lymphocytes: evidence for in situ t-cell activation and therapeutic efficacy. Jung G; Brandl M; Eisner W; Fraunberger P; Reifenberger G; Schlegel U; Wiestler O D; Reulen H J; Wilmanns W. (Department of Hematology and Oncology, Klinikum Grosshadern, University of Munich, Munich, Germany.. GundramJung@gmx.de) . INTERNATIONAL JOURNAL OF CANCER, (2001 Jan 15) 91 (2) 225-30. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB After adoptive transfer of pre-activated lymphocytes into the operation cavity of glioma patients, tumor regression and improved survival have been reported in some patients. Results were most impressive when **bispecific** antibodies with tumor x CD3 specificity were also applied. In this study, we attempted to avoid time-consuming pre-activation procedures for adoptively transferred cells by using a combination of **bispecific** antibodies directed to the **EGF receptor** (EGFR) on tumor cells and to CD3 and CD28 on T cells. Eleven patients with high-grade malignant glioma received 3 injections of 2 **bispecific** antibody fragments (EGFR x CD3 and EGFR x CD28) together with freshly isolated autologous lymphocytes via an Ommaya reservoir. Intracavitary fluid aspirated during immunotherapy was examined for markers of T-cell activation. Increased levels of soluble IL-2 receptor and TNF-alpha were detected in the intracavitary fluid of all patients tested. Two of the 11 treated patients experienced a beneficial response to therapy as defined by a transient contrast enhancement in subsequent MRI scans and prolonged survival. Side effects were transient and consisted of fever, nausea, headache and aggravation of pre-existing neurologic deficits. These adverse effects were most likely due to the antibody construct containing anti-CD3 specificity. Two patients developed cerebral edema and required steroid treatment.

L18 ANSWER 8 OF 27 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:157342 Document No.: PREV200100157342. Selection of cell binding and internalizing epidermal growth factor receptor antibodies from a phage display library. Heitner, Tara; Moor, Anne; Garrison, Jennifer L.; Marks, Cara; Hasan, Tayyaba; Marks, James D. (1). (1) Departments of Anesthesia and Pharmaceutical Chemistry, University of California, San Francisco, San Francisco General Hospital, 1001 Potrero Avenue, Room 3C-38, San Francisco, CA, 94110: marksj@anesthesia.ucsf.edu USA. Journal of Immunological Methods, (1 February, 2001) Vol. 248, No. 1-2, pp. 17-30. print. ISSN: 0022-1759. Language: English. Summary Language: English.

AB The first step in developing a targeted cancer therapeutic is generating a ligand that binds to a receptor which is either tumor specific or sufficiently overexpressed in tumors to provide targeting specificity. For this work, we generated human monoclonal antibodies to the **EGF receptor** (EGFR), an antigen overexpressed on many solid tumors. Single chain Fv (scFv) antibody fragments were directly selected by panning a phage display library on tumor cells (A431) overexpressing EGFR or Chinese hamster ovary cells (CHO/EGFR cells) transfected with the EGFR gene and recovering endocytosed phage from within the cell. Three unique scFvs were isolated, two from selections on A431 cells and two from

selections on CHO/EGFR cells. All three scFv bound native receptor as expressed on a panel of tumor cells and did not bind EGFR negative cells. Phage antibodies and multivalent immunoliposomes constructed from scFv were endocytosed by EGFR expressing cells as shown by confocal microscopy. Native scFv primarily stained the cell surface, with less staining intracellularly. The results demonstrate how phage antibodies binding native cell surface receptors can be directly selected on overexpressing cell lines or transfected cells. Use of a transfected cell line allows selection of antibodies to native receptors without the need for protein expression and purification, significantly speeding the generation of targeting antibodies to genomic sequences. Depending upon the format used, the antibodies can be used to deliver molecules to the cell surface or intracellularly.

L18 ANSWER 9 OF 27 MEDLINE DUPLICATE 3
2000387890 Document Number: 20347350. PubMed ID: 10888627. Ectodomain of coxsackievirus and adenovirus receptor genetically fused to epidermal growth factor mediates adenovirus targeting to epidermal growth factor receptor-positive cells. Dmitriev I; Kashentseva E; Rogers B E; Krasnykh V; Curiel D T. (Division of Human Gene Therapy, Departments of Medicine, Pathology, and Surgery, Gene Therapy Center, University of Alabama at Birmingham, Birmingham, Alabama 35294-3300, USA.) JOURNAL OF VIROLOGY, (2000 Aug) 74 (15) 6875-84. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Human adenovirus (Ad) is extensively used for a variety of gene therapy applications. However, the utility of Ad vectors is limited due to the low efficiency of Ad-mediated gene transfer to target cells expressing marginal levels of the Ad fiber receptor. Therefore, the present generation of Ad vectors could potentially be improved by modification of Ad tropism to target the virus to specific organs and tissues. The fact that coxsackievirus and adenovirus receptor (CAR) does not play any role in virus internalization, but functions merely as the virus attachment site, suggests that the extracellular part of CAR might be utilized to block the receptor recognition site on the Ad fiber knob domain. We proposed to design **bispecific** fusion proteins formed by a recombinant soluble form of truncated CAR (sCAR) and a targeting ligand. In this study, we derived sCAR genetically fused with human epidermal growth factor (EGF) and investigated its ability to target Ad infection to the **EGF receptor** (EGFR) overexpressed on cancer cell lines. We have demonstrated that sCAR-EGF protein is capable of binding to Ad virions and directing them to EGFR, thereby achieving targeted delivery of reporter gene. These results show that sCAR-EGF protein possesses the ability to effectively retarget Ad via a non-CAR pathway, with enhancement of gene transfer efficiency.

L18 ANSWER 10 OF 27 SCISEARCH COPYRIGHT 2002 ISI (R)
2000:882198 The Genuine Article (R) Number: 374LX. Construction and characterization of **bispecific** costimulatory molecules containing a minimized CD86 (B7-2) domain and single-chain antibody fragments for tumor targeting. Rohrbach F; Gerstmayer B; Biburger M; Wels W (Reprint). GEORG SPEYER HAUS, CHEMOTHERAPEUT FORSCHUNGSINST, PAUL EHRLICH STR 42-44, D-60596 FRANKFURT, GERMANY (Reprint); GEORG SPEYER HAUS, CHEMOTHERAPEUT FORSCHUNGSINST, D-60596 FRANKFURT, GERMANY; MEMOREC STOFFEL GMBH, D-50829 COLOGNE, GERMANY. CLINICAL CANCER RESEARCH (NOV 2000) Vol. 6, No. 11, pp. 4314-4322. Publisher: AMER ASSOC CANCER RESEARCH. PO BOX 11806, BIRMINGHAM, AL 35202. ISSN: 1078-0432. Pub. country: GERMANY. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Efficient T-cell activation requires two signals. The first signal, which confers specificity, is provided hy interaction of the T-cell receptor with peptides presented by MHC molecules, One of the second costimulatory signals is induced by binding of B7 proteins on the surface of antigen-presenting cells to CD28 on the T-cell surface. Expression of

B7 molecules on tumor cells can result in the activation of tumor specific T lymphocytes and induce protective antitumor immunity. However, at present such gene-therapeutic approaches are limited by the inability to selectively target B7 gene expression to cancer cells, As an alternative approach we exploited recombinant antibody fragments to localize a costimulatory B7 molecule to the surface of tumor cells. We constructed chimeric proteins that contain in a single polypeptide chain a portion of human B7-2 (CD86) genetically fused to single-chain (sc) Fv antibody domains specific for the tumor-associated antigens epidermal growth factor receptor and the closely related ErbB2 receptor tyrosine kinase, A small recombinant fragment of human CD86 was characterized that corresponds to amino acid residues 1-111 (CD86(111)) of the mature protein. CD86(111) produced in the yeast Pichia pastoris and CD86(111) expressed in bacteria was functionally active and displayed specific binding to B7 counter receptors, Bacterially expressed CD86(111)-scFv fusion proteins specifically localized to the respective target antigens on the surface of tumor cells and markedly enhanced the proliferation of primary T cells when bound to immobilized tumor antigen.

L18 ANSWER 11 OF 27 MEDLINE DUPLICATE 4

1999293471 Document Number: 99293471. PubMed ID: 10365137. Preclinical studies combining **bispecific** antibodies with cytokine-stimulated effector cells for immunotherapy of renal cell carcinoma. Elsasser D; Stadick H; Stark S; Van de Winkel J G; Gramatzki M; Schrott K M; Valerius T; Schafhauser W. (Department of Urology, Erlangen University of Erlangen-Nuremberg, Germany.. david.elsaesser@rzmail.uni-erlangen.de) . ANTICANCER RESEARCH, (1999 Mar-Apr) 19 (2C) 1525-8. Journal code: 8102988. ISSN: 0250-7005. Pub. country: Greece. Language: English.

AB BACKGROUND: **Bispecific** antibodies--consisting of a F(ab')-fragment derived from a monoclonal antibody against a tumor epitope as well as of another antibody against a cytotoxic trigger molecule on immune effector cells--can improve the effectiveness of antibody-based tumor therapy. MATERIALS AND METHODS: We used **bispecific** antibodies with one specifity against the **EGF-receptor** , which is overexpressed on the majority of renal cell carcinomas, and another specifity against Fc receptors on human leukocytes (Fc gamma RI/CD64; Fc gamma RIII/CD16 and Fc alpha RI/CD89). As source of effector cells, whole blood from patients treated with G-CSF, GM-CSF or IL2/IFN-alpha was used in 51Cr- release assays using various renal cancer cell lines as tumor targets. Further experiments with Percoll-isolated granulocytes or mononuclear cells from the same donors were performed in order to identify the active effector cell populations. RESULTS: Compared with conventional monoclonal EGF-R directed antibodies (murine IgG2a, humanized IgG1), **bispecific** antibodies induced significantly enhanced cytotoxicity. Highest amounts of tumor cell killing were observed using whole blood from patients treated with G-CSF or GM-CSF in combination with an [Fc alpha RI x EGF-R] **bispecific** antibody. Under these conditions, granulocytes constituted the most active effector cell population. CONCLUSION: The combination of myeloid growth factors and **bispecific** antibodies offer a promising new approach for the treatment of advanced renal cell carcinoma.

L18 ANSWER 12 OF 27 MEDLINE DUPLICATE 5

1999131864 Document Number: 99131864. PubMed ID: 9935165. Role of target antigen in **bispecific**-antibody-mediated killing of human glioblastoma cells: a pre-clinical study. Pfosser A; Brandl M; Salih H; Grosse-Hovest L; Jung G. (Department of Hematology and Oncology, Klinikum Grosshadern, University of Munich, Germany.) INTERNATIONAL JOURNAL OF CANCER, (1999 Feb 9) 80 (4) 612-6. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB **Bispecific** antibodies (bsAbs) directed to tumor-associated antigens and to receptors mediating T-cell activation, such as the TCR/CD3 complex and the co-stimulatory CD28 molecule, are capable of activating T

cells at the surface of tumor cells, resulting in tumor-cell killing. Here we report the pre-clinical characterization of **bispecific** -antibody fragments (bsFab2) directed to 2 different glioblastoma-associated antigens: the **EGF receptor** (EGFR) and a chondroitin-sulfate proteoglycan (CSPG). Using cultured glioblastoma cells expressing both target antigens, we found that the ability of anti-tumor x anti-CD28 bsFab2 to mediate "targeted T-cell co-stimulation" is superior for constructs targeting the CSPG molecule, correlating with an approximately 6-fold higher expression level of this antigen on the cell surface. In contrast, bsFab2 triggering CD3 are more effective if they contain EGFR-target specificity. This indicates that the activity of anti-tumor x anti-CD3 constructs critically depends on properties of the antigen other than its expression level on the cell surface, e.g., its mobility in the membrane. These findings prompted us to use EGFR-targeting bsFab2 in an ongoing clinical trial with glioma patients.

L18 ANSWER 13 OF 27 CAPLUS COPYRIGHT 2002 ACS
1999:2909 Document No. 130:149078 Differential susceptibility of primary and established human glioma cells to adenovirus infection: targeting via the epidermal growth factor receptor achieves fiber receptor-independent gene transfer. Miller, C. Ryan; Buchsbaum, Donald J.; Reynolds, Paul N.; Douglas, Joanne T.; Gillespie, G. Yancey; Mayo, Matthew S.; Raben, David; Curiel, David T. (Gene Therapy Program, University of Alabama at Birmingham, Birmingham, AL, 35294, USA). Cancer Research, 58(24), 5738-5748 (English) 1998. CODEN: CNREA8. ISSN: 0008-5472. Publisher: AACR Subscription Office.

AB Adenovirus (Ad) vectors are promising for gene therapy of glioma due to their ability to achieve efficient gene transfer upon intratumoral administration. Yet in this context, Ad mediates widespread gene transfer to both tumor and surrounding parenchyma. Ad entry is dependent upon the expression of fiber receptors, such as coxsackie/adenovirus receptor, and .alpha.v integrins on the target cells for binding and internalization, resp. We hypothesized that the susceptibility of human gliomas to Ad would likely be heterogeneous due to variable expression of these receptors. It was found that established human glioma cell lines exhibited differential susceptibility to Ad-mediated gene transfer, which correlated directly with the level of radiolabeled Ad binding and with the expression of coxsackie/adenovirus receptor but not with the expression of .alpha.v integrins. To circumvent the lack of fiber receptors and to target Ad gene transfer specifically to tumor cells, we used a **bispecific** antibody conjugate to ablate Ad binding to fiber receptors and retarget binding to the epidermal growth factor receptor (EGFR), a tumor-assocd. marker negligibly expressed in normal, mitotically quiescent neural tissues. The results demonstrate that EGFR-targeted Ad gene transfer was EGFR specific and independent of fiber-fiber receptor interactions. Furthermore, EGFR targeting significantly enhanced Ad gene delivery to 7 of 12 established glioma cell lines and to 6 of 8 cultured primary gliomas. Interestingly, EGFR-targeted Ad gene transfer did not correlate with EGFR expression across cell lines, suggesting the importance of other factors. This study establishes that fiber receptor expression limits the utility of Ad vectors for gene transfer to glioma cells and suggests that targeting Ad via EGFR may prove valuable for tumor-specific gene transfer to high-grade gliomas. These findings have key relevance in the context of Ad vector-based approaches for glioma gene therapy.

L18 ANSWER 14 OF 27 MEDLINE DUPLICATE 6
1998149664 Document Number: 98149664. PubMed ID: 9490020. The first constant domain (C(H)1 and C(L)) of an antibody used as heterodimerization domain for **bispecific** miniantibodies. Muller K M; Arndt K M; Strittmatter W; Pluckthun A. (Biochemisches Institut, Universitat Zurich, Switzerland.) FEBS LETTERS, (1998 Jan 30) 422 (2) 259-64. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB **Bispecific** miniantibodies were constructed by genetically fusing the C(H)1 domain of an IgG1 to the C-terminus of a single-chain Fv fragment (scFv-425), specific for the **EGF receptor,** and fusing the C(L) domain of a kappa light chain to the C-terminus of a scFv specific for CD2 (scFv-M1). An efficient dicistronic gene arrangement for functional expression in Escherichia coli was constructed. Immunoblots demonstrated correct domain assembly and the formation of the natural C(H)1-C(L) disulfide bridge. Gel filtration confirmed the correct size, sandwich ELISAs demonstrated **bispecific** functionality, and SPR biosensor measurements determined binding to EGF-R in comparison to bivalent constructs. **Bispecific** anti-EGF-R/anti-CD2 miniantibodies are candidates for the immunotherapy of cancer.

L18 ANSWER 15 OF 27 MEDLINE DUPLICATE 7
1998374022 Document Number: 98374022. PubMed ID: 9710248. A dimeric **bispecific** miniantibody combines two specificities with avidity. Muller K M; Arndt K M; Pluckthun A. (Biochemisches Institut, Universitat Zurich, Switzerland.) FEBS LETTERS, (1998 Jul 31) 432 (1-2) 45-9. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB **Bispecific** antibodies extend the capabilities of nature and might be applied in immunotherapy and biotechnology. By fusing the gene of a single-chain Fv (scFv) fragment to a helical dimerization domain, followed by a second scFv fragment of different specificity, we were able to express a functional protein in E. coli, which is **bispecific** and has two valencies for each specificity. The dimeric **bispecific** (DiBi) miniantibody preserves the natural avidity of antibodies in a very small-sized molecule of only 120 kDa. The generality of the principle was shown with a scFv fragment binding the **EGF-receptor** (named scFv 425) in three combinations with scFv fragments either directed against CD2 (ACID2.M1), phosphorylcholine (McPC603) or fluorescein (FITC-E2). Binding was analyzed by sandwich surface plasmon resonance biosensor (BIAcore) measurements.

L18 ANSWER 16 OF 27 SCISEARCH COPYRIGHT 2002 ISI (R)
97:82651 The Genuine Article (R) Number: WC638. Cytolytic and cytostatic properties of an anti-human Fc gamma RI (CD64) x epidermal growth factor **bispecific** fusion protein. Goldstein J; Graziano R F; Sundarapandiyan K; Somasundaram C; Deo Y M (Reprint). MEDAREX INC, 1545 ROUTE 22 E, ANNANDALE, NJ 08801 (Reprint); MEDAREX INC, ANNANDALE, NJ 08801. JOURNAL OF IMMUNOLOGY (15 JAN 1997) Vol. 158, No. 2, pp. 872-879. Publisher: AMER ASSOC IMMUNOLOGISTS. 9650 ROCKVILLE PIKE, BETHESDA, MD 20814. ISSN: 0022-1767. Pub. country: USA. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB A **bispecific** fusion protein (H22-EGF) that binds simultaneously to the epidermal growth factor receptor (ECF-R) and to the high affinity receptor for the Fc portion of human lgG, Fc gamma RI (CD64), has been successfully constructed and expressed. For this construction, genomic DNA encoding the Fd fragment of humanized anti-Fc gamma RI mAb, H22, which binds Fc gamma RI at an epitope that is distinct from the Fc binding site, was fused to cDNA encoding human epidermal growth factor (EGF), a,natural ligand for EGF-R. The resulting H22Fd-EGF-expressing vector was transfected into a myeloma cell line that was transfected previously with a vector containing DNA encoding the H22 kappa-light chain. SDS-PAGE analysis of purified H22-EGF demonstrated that the fusion protein was secreted predominantly as H22Fab'-EGF monomer (approximate to 55 kDa), even though a free Cys residue exists in the hinge region of the H22 Fab' component. Using a novel **bispecific** flow cytometry-binding assay, we demonstrated that the purified **bispecific** fusion protein, H22-EGF, was able to bind simultaneously to soluble Fc gamma RI and EGF-R-expressing cells. H22-EGF inhibited the growth of EGF-R-overexpressing tumor cells and mediated dose-dependent cytotoxicity of these cells in the presence of Fc gamma

RI-bearing cytotoxic effector cells. These results suggest that this fusion protein may have therapeutic utility for EGF-R-overexpressing malignancies.

L18 ANSWER 17 OF 27 CAPLUS COPYRIGHT 2002 ACS
1998:23212 Document No. 128:100754 Targeting tumor cell destruction with CD64-directed **bispecific** fusion proteins. Graziano, Robert F.; Goldstein, Joel; Sundarapandiyan, Karuna; Somasundaram, Chezian; Keler, Tibor; Deo, Yashwant M. (Medarex Inc., Annandale, NJ, 08801, USA). Cancer Immunology Immunotherapy, 45(3/4), 124-127 (English) 1997. CODEN: CIIMDN. ISSN: 0340-7004. Publisher: Springer-Verlag.
AB A review with 27 refs. summarizing the authors' current work with 2 CD64-targeted fusion proteins, H22-EGF and H22-heregulin .beta. (H22-HRG). The H22-EGF fusion protein binds specifically to cells that overexpress **EGF receptor**, while H22-HRG is targeted to cells that overexpress HER2, HER3, and/or HER4.

L18 ANSWER 18 OF 27 CAPLUS COPYRIGHT 2002 ACS
1996:654026 Document No. 125:292469 Targeted inhibition of tumor cell growth by a **bispecific** single-chain toxin containing an antibody domain and TGF.alpha.. Schmidt, M.; Wels, W. (Tumour Biology Center, Institute Experimental Cancer Research, Freiburg/Br., D-79011, Germany). British Journal of Cancer, 74(6), 853-862 (English) 1996. CODEN: BJCAAI. ISSN: 0007-0920. Publisher: Stockton.
AB The construction and functional characterization of a novel **bispecific** recombinant toxin, scFv(FRP5)-TGF.alpha.-ETA (scFv = epidermal growth factor receptor (EGFR)-specific single-chain Fv domains and ETA = Pseudomonas exotoxin A) were reported. The fusion protein consists of the antigen-binding domain of the ErbB-2-specific monoclonal antibody (MAb), FRP5, and the natural EGFR ligand TGF.alpha. inserted at different positions in truncated Pseudomonas exotoxin A. ScFv(FRP5)-TGF.alpha.-ETA protein displayed binding to EGFR and ErbB-2, thereby inducing activation of the receptors, which was dependent on the cellular context and the level of EGFR and ErbB-2 expression. The **bispecific** mol. was cytotoxic in vitro for tumor cells expressing various levels of the target receptors. In vivo scFv(FRP5)-TGF.alpha.-ETA potently inhibited the growth of established A431 tumor xenografts in nude mice.

L18 ANSWER 19 OF 27 MEDLINE DUPLICATE 8
96184158 Document Number: 96184158. PubMed ID: 8621240. A bivalent single-chain antibody-toxin specific for ErbB-2 and the **EGF receptor**. Schmidt M; Hynes N E; Groner B; Wels W. (Institute for Experimental Cancer Research, Tumor Biology Center, Freiburg, Germany.) INTERNATIONAL JOURNAL OF CANCER, (1996 Feb 8) 65 (4) 538-46. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.
AB ErbB-2 and **EGF receptors** are often co-expressed in human tumors and have been shown to synergize in the transformation of cells in experimental model systems. Transactivation of ErbB-2 can occur via ligand-induced heterodimerization with **EGF receptor** or other members of the ErbB family of receptor tyrosine kinases. We have previously described the potent anti-tumoral activity of the monospecific single-chain antibody-toxins scFv(FRP5)-ETA and scFv(225)-ETA binding to, respectively, ErbB-2 and the **EGF receptor**. Here we report the construction and functional characterization of a novel bivalent, **bispecific** single-chain antibody-toxin, scFv2(FRP5/225)-ETA. The fusion protein consists of 2 scFv domains specific for ErbB-2 and the **EGF receptor** linked to a modified Pseudomonas exotoxin A. ScFv2(FRP5/225)-ETA displayed in vitro cell killing activity on tumor cells overexpressing either ErbB-2 or the **EGF receptor** similar to that of the monospecific toxins. It was more potent in vitro and in vivo in inhibiting the growth of tumor

cells expressing both receptors. Treatment of A431 cells with scFv2(FRP5/225)-ETA led to an increase in **EGF receptor** and ErbB-2 phosphotyrosine content, most likely via the induction of receptor heterodimers. This may explain the enhanced toxicity of the **bispecific** antibody-toxin.

L18 ANSWER 20 OF 27 CAPLUS COPYRIGHT 2002 ACS
1995:753691 Document No. 123:141740 Anti-EGF-R/anti-CD3 **bispecific** monoclonal antibody, method for its production and its use. Mele, Antonio; De Santis, Rita; Ferrer, Marsal Cristina (Menarini Ricerche Sud S.P.A., Italy). PCT Int. Appl. WO 9516037 A1 19950615, 36 pp. DESIGNATED STATES: W: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SI, SK, TJ, TT, UA, US, UZ, VN; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1994-EP3995 19941201. PRIORITY: IT 1993-FI246 19931201.

AB It is described an anti-EGF-R/anti-CD3 **bispecific** monoclonal antibody (bimAb) of hybrid isotype (IgG1/IgG2a). It is also described the construction of a hybrid hybridoma secreting such bimAb, and the purifn. of anti-EGF-R/anti-CD3 bimAb mols. from hybrid hybridoma performed by protein-A cation exchange chromatog. Said bimAb turned out to be useful against tumor cells showing the epidermal growth factor receptor (EGF-R+). In example, hybridoma producing monoclonal anti-CD3 antibody was raised and fused with hybridoma producing anti-**EGF receptor** antibody for prodn. of the **bispecific** antibody. The bimAb was purified with protein A chromatog. and cation exchange chromatog.

L18 ANSWER 21 OF 27 CAPLUS COPYRIGHT 2002 ACS
1994:268211 Document No. 120:268211 Monoclonal antibodies recognizing the epidermal growth factor receptor, cells and methods for their production and compositions containing them. Fernandez Ordonez, Alicia Calle; Mateo de Acosta del Rio, Cristina Maria; Macias Abraham, Amparo Emilia; Macineira Perez, Pedro Pablo; Tormo Bravo, Blanca Rosa; Perez Rodriguez, Rolando (Centro de Immunologia Molecular, Cuba). Eur. Pat. Appl. EP 586002 A2 19940309, 34 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE. (English). CODEN: EPXXDW. APPLICATION: EP 1993-202428 19930818. PRIORITY: CU 1992-10092 19920818; CU 1993-1793 19930301.

AB A monoclonal antibody which recognizes human epidermal growth factor (**EGF**) **receptor** inhibits EGF binding to the receptor, inhibits growth of EGF-dependent tumor cells, and in addn. has at least one of the following properties: (a) has antibody-dependent cellular cytotoxicity; (b) has complement-mediated cytotoxic activity; (c) is capable of producing a synergistic effect in inhibiting proliferation of tumor cells if combined with a ganglioside; (d) is capable of producing a synergistic effect in inhibiting proliferation of tumor cells if combined with a monoclonal antibody against EGF. The monoclonal antibody (or a deriv. of the monoclonal antibody selected from humanized, chimeric, reshaped, **bispecific**, conjugated, and anti-idiotypic forms of it, or a fragment of it) is useful in the treatment of malignant neoplasms or preneoplastic diseases. A compn., a process for prepg. the monoclonal antibody, use of it, and an immortalized cell producing it are also claimed. Hybridomas were selected and the monoclonal antibodies were characterized.

L18 ANSWER 22 OF 27 MEDLINE DUPLICATE 9
94248685 Document Number: 94248685. PubMed ID: 8191221. Relevance of antibody valency in **EGF receptor** modulation. Morelli D; Villa E; Tagliabue E; Perletti L; Villa M L; Menard S; Balsari A; Colnaghi M I. (Division of Experimental Oncology E, University of Milan, Italy.) SCANDINAVIAN JOURNAL OF IMMUNOLOGY, (1994 May) 39 (5) 453-8. Journal code: 0323767. ISSN: 0300-9475. Pub. country: ENGLAND: United

Kingdom. Language: English.

AB Binding characteristics of a monovalent **bispecific** monoclonal antibody (bsMoAb), which recognizes both epidermal growth factor receptor (EGF-R) and drug doxorubicin (DXR) were compared with those of the parental bivalent MoAb directed against the EGF-R binding site. Scatchard analysis indicated that both MoAbs bound to EGF-R-overexpressing A431 cells with the same affinity. In tracer amounts, both MoAbs also displayed the same capacity to be internalized after binding to the cell surface. However, when the MoAbs were used at saturating concentrations, down-modulation of the receptor was greater with the bivalent MoAb. The bivalent MoAb also inhibited proliferation of A431 cells both in vitro and in vivo whereas the bsMoAb was inhibitory only in vivo. These data suggest that MoAb bivalency is required for EGF-R down-modulation and in vitro cell growth inhibition.

L18 ANSWER 23 OF 27 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 10

94:156225 The Genuine Article (R) Number: MY413. MODULATION OF DRUG-INDUCED CYTOTOXICITY BY A **BISPECIFIC** MONOCLONAL-ANTIBODY THAT RECOGNIZES THE EPIDERMAL GROWTH-FACTOR RECEPTOR AND DOXORUBICIN. MORELLI D; SARDINI A; VILLA E; VILLA M L; MENARD S; COLNAGHI M I (Reprint); BALSARI A. IST NAZL TUMORI, VIA G VENEZIAN 1, I-20133 MILAN, ITALY (Reprint); IST NAZL TUMORI, I-20133 MILAN, ITALY; UNIV MILAN, SCH MED, DEPT IMMUNOL, I-20133 MILAN, ITALY. CANCER IMMUNOLOGY IMMUNOTHERAPY (MAR 1994) Vol. 38, No. 3, pp. 171-177. ISSN: 0340-7004. Pub. country: ITALY. Language: ENGLISH. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB A hybrid hybridoma secreting a **bispecific** hybrid mAb (bsmAb), which recognizes both the epidermal growth factor receptor (EGF-R) and the drug doxorubicin, was produced by somatic hybridization of two hybridomas. The bsmAb obtained was able to retarget doxorubicin cytotoxicity in vitro specifically on EGF-R-positive cells exerting at the same time an antidotal effect on cells that did not overexpress the EGF-R. Distribution studies in mice indicate that the bsmAb selectively delivers the drug to tumour cells and modifies doxorubicin biodistribution with a statistically significant decrease of drug concentration in the intestine, which is the main target of early anthracycline toxicity. In keeping with this finding is the remarkable antidotal activity exerted by bsmAb in mice treated with doxorubicin, which is proved by retardation in loss of body weight and mortality. The effectiveness on tumour growth of the mAb followed by the administration of doxorubicin appears to be equal to that of the drug alone; however, the bsmAb exerts a remarkable antidotal activity.

L18 ANSWER 24 OF 27 MEDLINE DUPLICATE 11

94075058 Document Number: 94075058. PubMed ID: 8253530. Targeting of T lymphocytes against **EGF-receptor**+ tumor cells by **bispecific** monoclonal antibodies: requirement of CD3 molecule cross-linking for T-cell activation. Ferrini S; Cambiaggi A; Sforzini S; Marciano S; Canevari S; Mezzanzanica D; Colnaghi M I; Grossi C E; Moretta L. (Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy.) INTERNATIONAL JOURNAL OF CANCER, (1993 Dec 2) 55 (6) 931-7. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB Targeting of T lymphocytes against epidermal growth-factor-receptor (EGF-R)+ tumor cells was achieved by constructing a hybrid hybridoma which secretes an anti-EGF-R/anti-CD3 **bispecific** monoclonal antibody (biMAb) of hybrid isotype (IgG1/IgG2a). Purification of biMAb molecules from parental anti-EGF-R and anti-CD3 MAbs was performed by protein-A chromatography. The purified biMAb was able to trigger the lysis of EGF-R+ tumor cell lines (A431, IGROV-1, MDA-468 and U-87) and of NIH-3T3 transfectants expressing human EGF-R by cytolytic T lymphocytes, but it was ineffective in the case of EGF-R-negative tumor targets. Normal EGF-R+ cells (keratinocytes and endometrial cells) were also susceptible to biMAb-targeted cytolysis. However, the amount of biMAb required to induce half-maximal cytolysis of tumor cells over-expressing the EGF-R molecule (A431) was considerably lower than that required to induce lysis of EGF-R+

tumor or normal cells which express EGF-R at considerably lower density. The ability of such biMAbs to deliver activation signals to T cells was evaluated by Ca++ mobilization and lymphokine production experiments. The soluble anti-EGF-R/anti-CD3 biMAb failed to induce intracellular Ca++ increases, which occurred only after cross-linking induced by an anti-mouse IgG antibody. Secretion of lymphokines (IFN-gamma, TNF-alpha and GM-CSF) was induced by contact of the biMAb-coated effector cells with the relevant tumor target, whereas the soluble biMAb was virtually ineffective. In addition, biMAb-coated effector cells retained the ability to recognize and to lyse EGF-R+ tumor cells for a prolonged period of time. Our data indicate that activation of effector cells targeted by biMAbs can only occur at the tumor site, where cross-linking of surface CD3 molecules is induced by contact with the tumor cells.

L18 ANSWER 25 OF 27 MEDLINE DUPLICATE 12
93107863 Document Number: 93107863. PubMed ID: 1335026. The efficiency of cell targeting by recombinant retroviruses depends on the nature of the receptor and the composition of the artificial cell-virus linker. Etienne-Julan M; Roux P; Carillo S; Jeanteur P; Piechaczyk M. (Laboratoire de Biologie Moleculaire, URA CNRS 1191 Genetique Moleculaire, Universite Montpellier II Sciences et Techniques du Languedoc, France.) JOURNAL OF GENERAL VIROLOGY, (1992 Dec) 73 (Pt 12) 3251-5. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Using streptavidin-bound antibodies specific for both viral and cell membrane epitopes, we have reported previously that human cells may be infected by murine ecotropic retroviruses through an interaction with major histocompatibility complex class I and class II antigens, and thus have demonstrated that cell targeting by recombinant retroviruses is feasible. We report here that (i) growth factor or hormone receptors, such as those for epidermal growth factor (EGF) and insulin, can also mediate infection of human cells; (ii) a biotinylated cytokine or hormone can substitute for the anti-cell antibody in **bispecific** antibody complexes, thus extending the versatility of the method; (iii) although yields are low in our assay, infection efficiency clearly appears to depend upon the biochemical composition of molecular bridges because bi-functional antibody complexes are more efficient than cytokine-antibody complexes in the case of the **EGF receptor**. Finally, our study indicates that different cell membrane molecules are not equally efficient in allowing infection of human cells because targeting of the transferrin, high density lipoprotein and galactose receptors, as well as that of various membrane glycoconjugates, by murine ecotropic retroviruses did not lead to the establishment of a proviral state.

L18 ANSWER 26 OF 27 CAPLUS COPYRIGHT 2002 ACS
1993:167190 Document No. 118:167190 Development of humanized **bispecific** antibodies reactive with cytotoxic lymphocytes and tumor cells overexpressing the HER2 protooncogene. Shalaby, M. Refaat; Shepard, H. Michael; Presta, Len; Rodrigues, Maria L.; Beverley, Peter C. L.; Feldmann, Marc; Carter, Paul (Dep. Cell Biol., Genentech, Inc., South San Francisco, CA, 94080, USA). Journal of Experimental Medicine, 175(1), 217-25 (English) 1992. CODEN: JEMEAV. ISSN: 0022-1007.
AB The HER2 protooncogene encodes a 185-kD transmembrane phosphoglycoprotein, human epidermal growth factor receptor 2 (p185HER2), whose amplified expression on the cell surface can lead to malignant transformation. Overexpression of HER2/p185HER2 is strongly correlated with progression of human ovarian and breast carcinomas. Recent studies have shown that human T cells can be targeted with **bispecific** antibody to react against human tumor cells in vitro. Here, a **bispecific** F(ab')2 antibody mol. was developed consisting of a humanized arm with a specificity to p185HER2 linked to another arm derived from a murine anti-CD3 monoclonal antibody cloned from the UCHT1 hybridoma. The antigen-binding loops for the anti-CD3 were installed in the context of human variable region framework residues, thus forming a fully humanized

BsF(ab')2 fragment. Addnl. variants were produced by replacement of amino acid residues located in light chain complementarity detg. region 2 and heavy chain framework region 3 of the humanized anti-CD3 arm. Flow cytometry anal. showed that the **bispecific** F(ab')2 mols. can bind specifically to cells overexpressing p185HER2 and to normal human peripheral blood mononuclear cells bearing the CD3 surface marker. In addnl. expts., the presence of **bispecific** F(ab')2 caused up to fourfold enhancement in the cytotoxic activities of human T cells against tumor cells overexpressing p185HER2 as detd. by a 51Cr release assay. These **bispecific** mols. have a potential use as therapeutic agents for the treatment of cancer.

L18 ANSWER 27 OF 27 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1992:61227 Document No.: BR42:25127. PRODUCTION AND CHARACTERIZATION OF **BISPECIFIC** MONOCLONAL ANTIBODIES BSMABS RECOGNIZING THE **EGF-RECEPTOR** EGF-R AND DOXORUBICIN DXR. SARDINI A; VILLA E; MENARD S; COLNAGHI M I; BALSARI A. ONCOLOGIA SPERIMENTALE E, ISTITUTO NAZIONALE TUMORI, MILANO.. ELEVENTH BIENNIAL MEETING OF THE EUROPEAN ASSOCIATION FOR CANCER RESEARCH, GENOA, ITALY, NOVEMBER 3-6, 1991. EUR J CANCER. (1991) 27 (SUPPL 3), S56. CODEN: EJCAEL. ISSN: 0959-8049. Language: English.

=> s bispecific and Her-2
L21 125 BISPECIFIC AND HER-2

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L22 ANSWER 1 OF 56 SCISEARCH COPYRIGHT 2002 ISI (R)
2002:870785 The Genuine Article (R) Number: 605TP. Visualization of effective tumor targeting by CD8+natural killer T cells redirected with **bispecific** antibody F(ab ')(2)HER2xCD3. Scheffold C; Kornacker M; Scheffold Y C; Contag C H; Negrin R S (Reprint). Stanford Univ, Sch Med, Div Bone Marrow Transplantat, 300 Pasteur Dr, Stanford, CA 94305 USA (Reprint); Stanford Univ, Sch Med, Div Bone Marrow Transplantat, Stanford, CA 94305 USA; Stanford Univ, Sch Med, Dept Pediat, Stanford, CA 94305 USA. CANCER RESEARCH (15 OCT 2002) Vol. 62, No. 20, pp. 5785-5791. Publisher: AMER ASSOC CANCER RESEARCH. PO BOX 11806, BIRMINGHAM, AL 35202 USA. ISSN: 0008-5472. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB HER2 is an attractive immunotherapeutic target for neoplastic disease because this cell surface molecule is overexpressed on a large fraction of malignant tumor cells. To directly assess therapeutic responses to targeted therapy by noninvasive in vivo imaging in small animals, human HER2-expressing ovarian carcinoma cells were genetically modified with a firefly luciferase gene, and light emission was used for visualization of tumor growth and response to therapy. This imaging approach was able to demonstrate in real-time tumor regression in a HER2 xenograft mouse model by adoptive transfer of in vitro induced and expanded cytotoxic CD8+ natural killer T (NKT) cells retargeted with a humanized **bispecific** antibody F(ab')(2)HER2xCD3. Immunotherapy with effector cells alone or a humanized monoclonal antibody anti-p185(HER2) (4D5-8) resulted in significant but slower reduction in tumor burden. Long-term survival of tumor xenografts correlated inversely with visible residual tumor burden. In vitro, F(ab')2HER2xCD3 substantially augmented cytotoxic activity of CD8+ NKT cells. By flow-sorting, CD8+ NKT cells coexpressing CD56 were found to have the highest redirected killing ability. Treatment with concanamycin A or EGTA abrogated CD8+ NKT cytotoxicity indicating that perforin is a major pathway of tumor cell lysis. In contrast, when

CD8+ NKT cell were cross-linked with F(ab')(2)HER2xCD3 neither the immunosuppressants cyclosporine A and FK506, nor the increase of intracellular cyclic AMP by dibutyryl cyclic AMP were able to inhibit cytotoxicity demonstrating that signaling via the CD3 antigen changes the biological activity of non-MHC-restricted effector cells. These studies have demonstrated that CD8+ NKT cells can be successfully redirected to tumor cells using **bispecific** antibodies and offer a promising strategy for adoptive immunotherapy of neoplastic diseases.

L22 ANSWER 2 OF 56 SCISEARCH COPYRIGHT 2002 ISI (R)
2002:277497 The Genuine Article (R) Number: 534CE. Intracellular domains of target antigens influence their capacity to trigger antibody-dependent cell-mediated cytotoxicity. Tiroch K; Stockmeyer B; Frank C; Valerius T (Reprint). Univ Erlangen Nurnberg, Dept Med 3, Div Hematol Oncol, Krankenhausstr 12, D-91054 Erlangen, Germany (Reprint); Univ Erlangen Nurnberg, Dept Med 3, Div Hematol Oncol, D-91054 Erlangen, Germany. JOURNAL OF IMMUNOLOGY (1 APR 2002) Vol. 168, No. 7, pp. 3275-3282. Publisher: AMER ASSOC IMMUNOLOGISTS. 9650 ROCKVILLE PIKE, BETHESDA, MD 20814 USA. ISSN: 0022-1767. Pub. country: Germany. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Ab-mediated signaling In tumor cells and Ab-dependent cell-mediated cytotoxicity (ADCC) are both considered as relevant effector mechanisms for Abs in tumor therapy. To address potential interactions between these two mechanisms, we generated **HER-2**/neu- and CD19-derived chimeric target Ags, which were expressed in experimental tumor target cells. **HER-2**/neu-directed Abs were documented to mediate effective ADCC with both mononuclear cells (MNCs) and polymorphonuclear granulocytes (PMNs), whereas Abs against CD19 were effective only with MNCs and not with PMNs. We generated cDNA encoding **HER-2**/ CD19 or CD19/**HER-2** (extracellular/intracellular) chimeric fusion proteins by combining cDNA encoding extracellular domains of **HER-2**/neu or CD19 with intracellular domains of CD19 or **HER-2**/neu, respectively. After transfecting wild-type **HER-2**/neu or chimeric **HER-2**/CD19 into Raji Burkitt's lymphoma cells and wild-type CD19 or chimeric CD19/**HER-2** into SK-BR-3 breast cancer cells, target cell lines were selected for high membrane expression of transfected Ags. We then investigated the efficacy of tumor cell lysis by PMNs or MNCs with CD19- or **HER-2** /neu-directed Ab constructs. MNCs triggered effective ADCC against target cells expressing wild-type or chimeric target Ag. As expected, PMNs killed wild-type **HER-2**/neu-transfected, but not wild-type CD19-transfected target cells. Interestingly, however, PMNs were also effective against chimeric CD19/**HER-2**-transfected, but not **HER-2**/CD19-transfected target cells. In conclusion, these results demonstrate that intracellular domains of target Ags contribute substantially to effective Ab-mediated tumor cell killing by PMNs.

L22 ANSWER 3 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2002:396116 Document No.: PREV200200396116. A new class of trifunctional **bispecific** antibodies mediates efficient removal of occult metastatic cells (OMC. Prang, Nadja S. (1); Schoberth, Alexandra; Braun, Stephan; Janni, Wolfgang; Lindhofer, Horst. (1) Laboratory for Molecular Oncology, Munich Germany. Proceedings of the American Association for Cancer Research Annual Meeting, (March, 2002) Vol. 43, pp. 751. print. Meeting Info.: 93rd Annual Meeting of the American Association for Cancer Research San Francisco, California, USA April 06-10, 2002 ISSN: 0197-016X. Language: English.

L22 ANSWER 4 OF 56 MEDLINE DUPLICATE 1
2002083005 Document Number: 21668038. PubMed ID: 11809717. Adenovirus targeting to c-erbB-2 oncoprotein by single-chain antibody fused to

trimeric form of adenovirus receptor ectodomain. Kashentseva Elena A; Seki Toshiro; Curiel David T; Dmitriev Igor P. (Division of Human Gene Therapy, Department of Medicine, University of Alabama at Birmingham, 35294-3300, USA.) CANCER RESEARCH, (2002 Jan 15) 62 (2) 609-16. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB The use of adenovirus (Ad) vectors for cancer gene therapy applications is currently limited by several factors, including broad Ad tropism associated with the widespread expression of coxsackievirus and adenovirus receptor (CAR) in normal human tissues, as well as limited levels of CAR in tumor cells. To target Ad to relevant cell types, we have proposed using soluble CAR (sCAR) ectodomain fused with a ligand to block CAR-dependent native tropism and to simultaneously achieve infection through a novel receptor overexpressed in target cells. To confer Ad targeting capability on cancer cells expressing the c-erbB-2/**HER-2**/neu oncogene, we engineered a **bispecific** adapter protein, sCARfC6.5, that consisted of sCAR, phage T4 fibritin polypeptide, and C6.5 single-chain fragment variable (scFv) against c-erbB-2 oncoprotein. Incorporation of fibritin polypeptide provided trimerization of sCAR fusion proteins that, compared with monomeric sCAR protein, resulted in augmented affinity to Ad fiber knob domain and in increased ability to block CAR-dependent Ad infection. We demonstrated that sCARfC6.5 protein binds to cellular c-erbB-2 oncoprotein and mediates efficient Ad targeting via a CAR-independent pathway. As illustrated in cancer cell lines that overexpress c-erbB-2, targeted Ad, complexed with sCARfC6.5 adapter protein, provided from 1.5- to 17-fold enhancement of gene transfer compared with Ad alone and up to 130-fold increase in comparison with untargeted Ad complexed with sCARf control protein. The use of recombinant trimeric sCAR-scFv adapter proteins may augment Ad vector potency for targeting cancer cell types.

L22 ANSWER 5 OF 56 MEDLINE DUPLICATE 2

2002318050 Document Number: 21972410. PubMed ID: 11976831. The pharmacokinetics of the **bispecific** antibody MDX-H210 when combined with interferon gamma-1b in a multiple-dose phase I study in patients with advanced cancer. Lewis Lionel D; Beelen Andrew P; Cole Bernard F; Wallace Paul K; Fisher Jan L; Waugh Mary G; Kaufman Peter A; Ernstoff Marc S. (Department of Medicine, Dartmouth Medical School and The Norris Cotton Cancer Center, Lebanon, NH 03756, USA.. Lionel.D.Lewis@Dartmouth.edu) . CANCER CHEMOTHERAPY AND PHARMACOLOGY, (2002 May) 49 (5) 375-84. Journal code: 7806519. ISSN: 0344-5704. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB INTRODUCTION: MDX-H210 is a Fab'xFab' **bispecific** antibody (BsAb) constructed chemically by crosslinking Fab' mAb 520C9 (anti-**HER-2**/neu) and humanized Fab' mAbH22 (anti-CD64). STUDY DESIGN AND OBJECTIVES: This was a phase I dose-escalation study of intravenous MDX-H210 (1-70 mg/m(2)) combined with subcutaneous IFN-gamma, 50 microg/m(2) given 24 h before the BsAb, both drugs being given three times a week for 3 weeks. The major objectives of the study were to define the safety, tolerability and pharmacokinetics of MDX-H210 when given with IFN-gamma on this schedule. RESULTS: The study group comprised 23 patients (19 female, 4 male; median age 51.5 years, range 25-72 years) with advanced **HER-2**/neu-positive cancers (19 breast, 3 prostate and 1 lung). Inspection of the log plasma MDX-H210 concentrations-time data for both days 1 and 17 of treatment revealed monoexponential decay in the majority of patients with adequate concentration-time data points. The MDX-H210 T(1/2) ranged from 2.9 to 21.9 h. The MDX-H210 C(max) on day 1 (means+/-SD) increased from 0.30+/-0.22 microg/ml at the 1-mg/m(2) dose tier to 86.91+/-6.46 microg/ml at 70 mg/m(2). Equivalent day-17 values were 0.27+/-0.30 microg/ml increasing to 147.85+/-40.23 microg/ml. The MDX-H210 T(max) occurred at or after the end of the infusion for all treatments. The mean MDX-H210 total body clearance (Cl) was in the range 0.01-0.34 ml/min per kg and the mean MDX-H210 apparent volume of distribution at steady-state (Vd(ss)) in the

range 20-170 ml/kg, compatible with distribution primarily limited to the intravascular space. MDX-H210 T(1/2) increased with dose (ANOVA P=0.001) and Cl decreased with dose (ANOVA P=0.006). There were no significant changes in MDX-H210 C(max), AUC, Cl or Vd(ss) between day 1 and day 17. CONCLUSIONS: MDX-H210 pharmacokinetics appeared saturable over the dose range 1-70 mg/m(2), and there was no significant change in MDXH210 pharmacokinetics over the course of the study, or evidence of excessive accumulation of MDX-H210 on this multiple dosing schedule. When MDX-H210 was combined with IFN-gamma, the estimated MDX-H210 pharmacokinetic parameters were similar to the published data for single-agent MDX-H210.

L22 ANSWER 6 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2002:227464 Document No.: PREV200200227464. Dose-dependent pharmacokinetics of the **bispecific** antibody MDX-H210. Beelen, A. P. (1); Cole, B. F. (1); Wallace, P. K. (1); Fisher, J. L. (1); Waugh, M. G. (1); Kaufman, P. A. (1); Ernstoff, M. S. (1); Nierenberg, D. W. (1); Lewis, L. D. (1). (1) Dartmouth Hitchcock Medical Center, Dartmouth Medical School, Lebanon, NH USA. Clinical Pharmacology & Therapeutics, (February, 2002) Vol. 71, No. 2, pp. P48. print. Meeting Info.: Annual Meeting of the American Society for Clinical Pharmacology and Therapeutics Atlanta, Georgia, USA March 24-27, 2002 ISSN: 0009-9236. Language: English.

L22 ANSWER 7 OF 56 MEDLINE DUPLICATE 3
2001376626 Document Number: 21326013. PubMed ID: 11433407.
Bispecific single-chain antibodies as effective tools for eliminating epithelial cancer cells from human stem cell preparations by redirected cell cytotoxicity. Maletz K; Kufer P; Mack M; Raum T; Pantel K; Riethmuller G; Gruber R. (Institute for Immunology, Ludwig-Maximilians-Universitat Munchen, Munich, Germany.) INTERNATIONAL JOURNAL OF CANCER, (2001 Aug 1) 93 (3) 409-16. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.
AB High-dose chemotherapy (HDC) with autologous bone marrow or peripheral stem cell transplantation is discussed as one option to treat the extensive stage of a variety of tumors. Effective methods to eliminate contaminating tumor cells from human bone marrow or stem cell grafts may improve the outcome of the patients. We investigated 3 recombinant **bispecific** single-chain antibodies (bscAbs) directed against 17-1A (EpCAM), c-erbB-2 (**HER-2**/neu) and LeY on the one and CD3 on the other binding site for their ability to induce lysis of epithelial tumor cells by retargeting autochthonous T lymphocytes present in bone marrow mononuclear cells (BMMC) and in peripheral stem cell mononuclear cells (PSMC). The bscAbs showed remarkable specific lysis of different epithelial tumor cell lines with BMMCs as well as with PSMCs as effector cells. Investigation of the alpha 17-1A-alpha CD3 bscAb revealed a significant correlation between the percentage of CD3(+) cells present in the BMMCs and the rate of lysis as well as the absence of detrimental effects on the viability of hematopoietic progenitor cells as determined by colony-forming unit assays (CFUs). Our results indicate that recombinant **bispecific** single-chain antibodies could be new tools for purging of human bone marrow and peripheral stem cell grafts from contaminating epithelial cancer cells for patients receiving autologous stem cell transplantation after HDC.

L22 ANSWER 8 OF 56 MEDLINE DUPLICATE 4
2001518309 Document Number: 21449517. PubMed ID: 11565839. Subcutaneous administration of interleukin-2 triggers Fcgamma receptor I expression on human peripheral blood neutrophils in solid and hematologic malignancies. Sconocchia G; Cococcetta N Y; Campagnano L; Amadori S; Iorio B; Boffo V; Ferdinandi V; Del Principe I; Adorno D; Casciani C U. (Institute of Tissue Typing and Dialysis, Consiglio Nazionale delle Ricerche, Rome, Italy.) JOURNAL OF IMMUNOTHERAPY, (2001 Jul-Aug) 24 (4) 374-83. Journal code: 9706083. ISSN: 1524-9557. Pub. country: United States. Language: English.

AB Freshly isolated human polymorphonuclear cells (PMNCs) constitutively express Fcgamma receptor (Fc-gammaR) II and FcgammaRIII on the cell surface but not FcgammaRI. Cytokines such as interferon-gamma (IFNgamma), granulocyte-macrophage colony-stimulating factor (CSF), and granulocyte-CSF trigger FcgammaRI expression on (PMNCs). Because PMNCs express interleukin (IL)-2 receptor, we investigated whether IL-2 can induce FcgammaRI expression on PMNCs isolated from IL-2-treated metastatic renal cell carcinoma (MRCC) and low-grade non-Hodgkin lymphoma (LGNHL) patients. Pretherapy flow cytometry analysis of Fcgamma receptors on PMNCs did not show FcgammaRI expression. Interestingly, 3 days after therapy, PMNCs displayed a detectable amount of FcgammaRI on the cell surface. Kinetic studies on the in vivo effects of IL-2 on MRCC patients showed that FcgammaRI was transiently expressed, starting within 3-6 days of therapy, remaining expressed for 10-15 days, and rapidly declining, whereas such expression remained stable for months in LGNHL patients. In contrast, Fc-gammaRII was not affected. In addition, FcgammaRI+ PMNCs coated in vitro with a **bispecific** antibody Fab anti-FcgammaRI x anti-**HER-2**/neu formed intercellular conjugates with a human **HER-2**/neu-transfected 3T3 cell line (**HER -2**/neu-3T3). Interleukin-2 treatment increased the number of FcgammaRIII low eosinophils, leading to a change in FcgammaRIII distribution among granulocyte cell subsets. In vitro IL-2 treatment of purified PMNCs failed to generate Fc-gammaRI expression, suggesting that IL-2 indirectly causes FcgammaRI expression. During the IL-2 administration, we did not observe significant changes in IFNgamma serum level. In conclusion, our observation may be used to potentiate the antitumor effects of IL-2 in novel immunotherapy regimens, perhaps by redirecting FcgammaRI+ PMNCs against cancer cells by heteroconjugate antibodies and monitoring the biologic activity of subcutaneous IL-2 in cancer patients.

L22 ANSWER 9 OF 56 SCISEARCH COPYRIGHT 2002 ISI (R)
2001:393259 The Genuine Article (R) Number: 429YK. Use of anti-CD3 x anti-HER2/neu **bispecific** antibody for redirecting cytotoxicity of activated T cells toward HER2/neu+ tumors. Sen M; Wankowski D M; Garlie N K; Siebenlist R E; Van Epps D; LeFever A V; Lum L G (Reprint). Roger Williams Med Ctr, N Campus, Room G01, 825 Chalkstone Ave, Providence, RI 02908 USA (Reprint); Roger Williams Med Ctr, Providence, RI 02908 USA; Nexell Therapeut Inc, Irving, CA 92618 USA; St Lukes Med Ctr, Immunotherapy Program, Milwaukee, WI 53201 USA; Blood Ctr SE Wisconsin, Milwaukee, WI 53201 USA. JOURNAL OF HEMATOTHERAPY & STEM CELL RESEARCH (APR 2001) Vol. 10, No. 2, pp. 247-260. Publisher: MARY ANN LIEBERT INC PUBL. 2 MADISON AVENUE, LARCHMONT, NY 10538 USA. ISSN: 1525-8165. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Relapse after adjuvant chemotherapy or high-dose chemotherapy with stem cell transplant for highrisk breast cancer remains high and new strategies that provide additional antitumor effects are needed. This report describes methods to generate highly effective HER2/neu-specific cytotoxic T cells by arming activated T cells with anti-CD3 x anti-HER2/neu **bispecific** antibody (BsAb). OKT3 and 9184 (anti-HER2) monoclonal antibodies (mAb) were conjugated and used to arm T cells that were subsequently tested in binding, cytotoxicity, and cytokine secretion assays. Armed T cells aggregated and specifically killed HER2/neu(+) breast cancer cells. Cytotoxicity emerged after 6 days of culture, was higher in armed T cells than unarmed T cells at all effector to target ratios (E/T) tested, and increased as the arming dose was increased. At an E/T of 20:1, the mean cytotoxicity of armed activated T cells (ATC) from 10 normal subjects increased by 59 +/- 11% (+/-SD) over that seen in unarmed ATC ($p < 0.001$) and the mean cytotoxicity of armed ATC from 6 cancer patients increased by 32 +/- 9% above that seen for unarmed ATC ($p < 0.0004$). After arming, the BsAb persisted on ATC up to 72 h and armed ATC continued to be cytotoxic up to 54 h. The amount of interferon-gamma

(IFN-gamma), tumor necrosis factor-alpha (TNF-alpha), and granulocyte-macrophage colony-stimulating factor (GM-CSF) secreted was 1699, 922, and 3092 pg/ml/10(6) cells per 24 h, respectively, when armed T cells were exposed to a HER2/neu(+) breast carcinoma cell line. These studies show the feasibility and clinical adaptability of this approach for generating large numbers of anti-HER2-specific, cytotoxic T cells for clinical trials.

L22 ANSWER 10 OF 56 MEDLINE DUPLICATE 5
2001196544 Document Number: 21126047. PubMed ID: 11223077.
Bispecific antibody-targeted phagocytosis of **HER-2**/neu expressing tumor cells by myeloid cells activated in vivo.
Wallace P K; Kaufman P A; Lewis L D; Keler T; Givan A L; Fisher J L; Waugh M G; Wahner A E; Guyre P M; Fanger M W; Ernstoff M S. (Department of Microbiology, HB7556, Dartmouth Medical School and the Immunology Immunotherapy Program of the Norris Cotton Cancer Center, 1 Medical Center Drive, Lebanon, NH 03756, USA.. pkw@dartmouth.edu) . JOURNAL OF IMMUNOLOGICAL METHODS, (2001 Feb 1) 248 (1-2) 167-82. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.
AB Studies from our laboratory and others have established that both mononuclear phagocytes and neutrophils mediate very efficient cytotoxicity when targeted through Fc receptors using a suitable monoclonal or **bispecific** antibody (BsAb). Cross-linking an Fc receptor for IgG (FcgammaR) triggers multiple anti-tumor activities including superoxide generation, cytokine and enzyme release, phagocytosis and antibody-dependent cellular cytotoxicity (ADCC). In this report, using unfractionated leukocytes and two color flow cytometric analysis, we describe the phagocytic capacity of peripheral blood polymorphonuclear cells (PMN) and monocytes isolated from patients enrolled in a phase I clinical trial of MDX-H210 given in combination with IFNgamma. MDX-H210 is a BsAb targeting the myeloid trigger molecule FcgammaRI and the **HER-2**/neu proto-oncogene product overexpressed on a variety of adenocarcinomas. In this trial, cohorts of patients received escalating doses of MDX-H210 3 times per week for 3 weeks. Interferon-gamma (IFNgamma) was given 24 h prior to each BsAb infusion. Our results demonstrate that monocytes from these patients were inherently capable of phagocytosing the **HER-2**/neu positive SK-BR-3 cell line and that addition of MDX-H210 into the assay significantly enhanced the number of targets phagocytosed. Two days after administration of an immunologically active dose of MDX-H210 (10 mg/m2), monocytes from these patients were able to phagocytose greater amounts of target cell material, indicating that these cells remained armed with functionally sufficient BsAb for at least 48 h. PMN from these patients very efficiently mediated phagocytosis through FcgammaRI after being treated with IFNgamma, but not before. We conclude that phagocytosis is not only an efficient mechanism of myeloid cell-mediated cytotoxicity, but may also be a mechanism by which antigens from phagocytosed cells can enter a professional antigen presenting cell for processing and presentation.

L22 ANSWER 11 OF 56 SCISEARCH COPYRIGHT 2002 ISI (R)
2001:619100 The Genuine Article (R) Number: 457TG. A phase II study of the **bispecific** antibody MDX-H210 (anti-HER2 x CD64) with GM-CSF in HER2+advanced prostate cancer. James N D (Reprint); Atherton P J; Jones J; Howie A J; Tchekmedyian S; Curnow R T. Univ Birmingham, CRC, Inst Canc Studies, Birmingham B15 2TA, W Midlands, England (Reprint); Univ Birmingham, Dept Pathol, Birmingham B15 2TT, W Midlands, England; Pacific Shores Med Grp, Long Beach, CA 90813 USA. BRITISH JOURNAL OF CANCER (20 JUL 2001) Vol. 85, No. 2, pp. 152-156. Publisher: CHURCHILL LIVINGSTONE. JOURNAL PRODUCTION DEPT, ROBERT STEVENSON HOUSE, 1-3 BAXTERS PLACE, LEITH WALK, EDINBURGH EH1 3AF, MIDLOTHIAN, SCOTLAND. ISSN: 0007-0920. Pub. country: England; USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The proto-oncogene HER2 presents a novel therapeutic target. We report results in 25 patients with HER2+ advanced prostate cancer treated with the **bispecific** antibody MDX-H210 15 mug m(-2) by intravenous infusion plus GM-CSF 5 mug kg(-1) day(-1) by subcutaneous injection for 4 days repeated weekly for 6 weeks. Patients with stable disease or better received further cycles of treatment until disease progression or study withdrawal. 1 patient received no treatment and 4 received less than 1 cycle and are included in the toxicity analysis only. Median duration of follow up was 105+ (range 21-188) days. Toxicity was generally NCI-CTG 0-2. There were 2 grade 4 adverse events (heart failure and dyspnoea) and 1 grade 3 event (allergic reaction) resulting in discontinuation of the study medication. There were 9 further grade 3 events not resulting in trial withdrawal. There were no treatment-related deaths. 7/20 (35%) evaluable patients had a >50% PSA response of median duration 128 (range 71-184+) days. 7/12 (58%) patients with evaluable pain had improvements in pain scores. The PSA relative velocity on therapy decreased in 15/18 (83%) assessable patients compared to pre-study. GM-CSF and MDX-H210 is active in hormone refractory prostate carcinoma with acceptable toxicity; further studies are warranted., (C) 2001 Cancer Research Campaign hftp://www.bjcancer.com.

L22 ANSWER 12 OF 56 MEDLINE DUPLICATE 6
2001159755 Document Number: 21126046. PubMed ID: 11223076.
Pharmacokinetic-pharmacodynamic relationships of the **bispecific** antibody MDX-H210 when administered in combination with interferon gamma: a multiple-dose phase-I study in patients with advanced cancer which overexpresses **HER-2**/neu. Lewis L D; Cole B F; Wallace P K; Fisher J L; Waugh M; Guyre P M; Fanger M W; Curnow R T; Kaufman P A; Ernstoff M S. (Department of Medicine, Dartmouth Medical School and The Norris Cotton Cancer Center, Lebanon, NH 03756, USA.. lionel.d.lewis@dartmouth.edu) . JOURNAL OF IMMUNOLOGICAL METHODS, (2001 Feb 1) 248 (1-2) 149-65. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB INTRODUCTION: MDX-H210 is a Fab'xFab' **bispecific** antibody (BsAb) constructed chemically by crosslinking Fab' mAb 520C9 (anti-**HER-2**/neu) and Fab' mAbH22 (anti-CD64). STUDY DESIGN AND OBJECTIVES: This was a dose escalation study of intravenous MDX-H210 (1-70 mg/m(2)), preceded 24 h beforehand by subcutaneous IFNgamma (50 microg/m(2) to up-regulate FcgammaRI) administered three times a week for 3 weeks. We investigated the pharmacokinetic-pharmacodynamic relationships between MDX-H210 C(max) and AUC and (i) MDX-H210 binding to peripheral blood monocytes and neutrophils, (ii) the peak plasma G-CSF, IL-6, IL-8 and TNFalpha concentrations, and (iii) the observed clinical toxicity. RESULTS: 23 patients (19F:4M; median age 51.5; range 25-72 y) with advanced **HER-2**/neu positive cancers (19 breast, three prostate and one lung) were studied. Plasma MDX-H210 concentrations over time, circulating numbers of monocytes and neutrophils, percent saturation of monocyte and neutrophil FcgammaRI, and plasma concentrations over time of G-CSF, IL-6, IL-8 and TNFalpha were measured and clinical toxicity monitored. The E(max) pharmacodynamic model best fitted the relationship of MDX-H210 C(max) and the maximum percent saturation of both monocytes (E(max)=74.6; EC(50)=0.9 microg/ml) and neutrophils (E(max)=66.2; EC(50)=2.3 microg/ml) on the first day of treatment. On the last day of treatment, day 19, these parameters were E(max)=57.0% and EC(50)=0.46 microg/ml for monocytes and E(max)=61.9% and EC(50)=0.26 microg/ml for neutrophils. No positive relationship was defined between the log MDX-H210 C(max) and the log peak plasma IL-6, G-CSF, TNF or IL-8 concentrations on day 1. On day 19 these plasma cytokine concentrations were undetectable post MDX-H210 therapy. There was no consistent relationship between MDX-H210 C(max) and the observed clinical toxicities. CONCLUSIONS: These data suggest that MDX-H210 C(max) and AUC could be related by the E(max) model to maximum percent FcgammaRI saturation on circulating monocytes and neutrophils in the patients studied. After day 1, the post MDX-H210

therapy cytokine response attenuated over time, consistent with desensitization. We did not find a relationship between log MDX-H210 C(max) and peak plasma cytokine concentrations or clinical toxicities.

L22 ANSWER 13 OF 56 MEDLINE DUPLICATE 7
2001196540 Document Number: 21126042. PubMed ID: 11223072. Mechanisms of G-CSF- or GM-CSF-stimulated tumor cell killing by Fc receptor-directed **bispecific** antibodies. Stockmeyer B; Elsasser D; Dechant M; Repp R; Gramatzki M; Glennie M J; van de Winkel J G; Valerius T. (Division of Hematology/Oncology, Department of Medicine III, University of Erlangen-Nurnberg, Krankenhausstrasse 12, D-91054, Erlangen, Germany.. bernhard.stockmeyer@med3.imed.uni-erlangen.de) . JOURNAL OF IMMUNOLOGICAL METHODS, (2001 Feb 1) 248 (1-2) 103-11. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB Studies with gene-modified mice have recently reinforced the importance of Fc receptor-mediated effector mechanisms for the therapeutic efficacy of rituxan and herceptin - two clinically approved antibodies for the treatment of tumor patients. We investigated Fc receptor-dependent tumor cell killing by mononuclear and granulocytic effector cells - comparing human IgG1 antibodies against CD20 or **HER-2**/neu with their respective FcgammaRI (CD64)-, FcgammaRIII (CD16)-, or FcalphaRI (CD89)-directed **bispecific** derivatives. With blood from healthy donors as effector source, human IgG1 and FcgammaRIII (CD16)-directed **bispecific** antibodies proved most effective in recruiting mononuclear effector cells, whereas tumor cell killing by granulocytes was most potently triggered by FcalphaRI-directed **bispecific** constructs. Granulocyte-mediated tumor cell lysis was significantly enhanced when blood from G-CSF- or GM-CSF-treated patients was investigated. Interestingly, however, both myeloid growth factors improved effector cell recruitment by different mechanisms, which were furthermore dependent on the tumor target antigen, and on the selected cytotoxic Fc receptor.

L22 ANSWER 14 OF 56 MEDLINE DUPLICATE 8
2001156012 Document Number: 21078533. PubMed ID: 11211151. Phase I pilot trial of the **bispecific** antibody MDXH210 (anti-Fc gamma RI X anti-**HER-2**/neu) in patients whose prostate cancer overexpresses **HER-2**/neu. Schwaab T; Lewis L D; Cole B F; Deo Y; Fanger M W; Wallace P; Guyre P M; Kaufman P A; Heaney J A; Schned A R; Harris R D; Ernstoff M S. (Uro-Oncology Program, Norris Cotton Cancer Center and Section of Urology and Immunology and Immunotherapy Research Programs, Dartmouth-Hitchcock Medical Center, Lebanon, New Hampshire, USA.) JOURNAL OF IMMUNOTHERAPY, (2001 Jan-Feb) 24 (1) 79-87. Journal code: 9706083. ISSN: 1524-9557. Pub. country: United States. Language: English.

AB The goal of this study was to evaluate, in patients with prostate cancer, the toxicity profile and biologic activity of the **bispecific** antibody MDXH210, which has specificity for the non-ligand-binding site of the high-affinity immunoglobulin G receptor (Fc gamma RI) and the extracellular domain of the **HER-2**/neu proto-oncogene product. Patients with prostate cancer that expressed **HER-2**/neu were entered into a phase I dose-escalation trial of MDXH210. Patients received an intravenous infusion MDXH210 during a period of 2 h three times per week for 2 weeks and were monitored for toxicity. Pharmacokinetic and pharmacodynamic parameters were measured and included the biologic end points of monocyte-bound MDXH210, cytokine production, and clinical response. Seven patients were treated with MDXH210 doses ranging from 1 to 8 mg/m2. In general, MDXH210 was well tolerated, with only mild infusion-related malaise, fever, chills, and myalgias. No dose-limiting toxic effects were observed. Biologic effects included induction of low plasma concentrations of tumor necrosis factor-alpha and interleukin-6 observed immediately after MDXH210 infusion and 70% saturation of circulating monocyte-associated Fc gamma RI with MDXH210 at

a dose level of 4 to 8 mg/m2. Five of six patients had stable prostate-specific antigen levels during the course of 40 days or more. Circulating plasma **HER-2**/neu levels decreased by 80% at days 12 and 29 (p = 0.03 and 0.06, respectively, by the Wilcoxon signed rank test). MDXH210 can be given safely to patients with **HER-2**/neu-positive prostate cancer in doses of at least 8 mg/m2. At the doses studied, biologic activity was demonstrated and characterized by binding of MDXH210 to circulating monocytes, release of monocyte-derived cytokines, a decrease in circulating **HER-2**/neu, and short-term stabilization of prostate-specific antigen levels.

L22 ANSWER 15 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:479948 Document No.: PREV200100479948. Targeting of prostate cancer with T cells armed with OKT3 x anti-**HER-2**/neu **bispecific** monoclonal antibodies (BiAbs. Lum, L. G. (1); Rathore, R.; Lum, H. E.; Miller, M.; Hachem, G.; Corvese, D. M.; Van Epps, D. E.; Tiller, H.; Elfenbein, G. J.. (1) Roger Williams Medical Center, Providence, RI USA. Experimental Hematology (Charlottesville), (August, 2001) Vol. 29, No. 8 Supplement 1, pp. 75. print. Meeting Info.: 30th Annual Meeting of the International Society for Experimental Hematology Tokyo, Japan August 25-28, 2001 ISSN: 0301-472X. Language: English. Summary Language: English.

L22 ANSWER 16 OF 56 CAPLUS COPYRIGHT 2002 ACS
2000:716534 Document No. 134:324726 **Bispecific** antibody MDX-210 for treatment of advanced ovarian and breast cancer. Kaufman, Peter A.; Wallace, Paul K.; Valone, Frank H.; Wells, Wendy A.; Memoli, Vincent A.; Ernstoff, Marc S. (Section of Hematology/Oncology, Dartmouth Hitchcock Medical Center, Lebanon, NH, USA). Methods in Molecular Medicine, 39(Ovarian Cancer), 793-806 (English) 2000. CODEN: MMMEFN. Publisher: Humana Press Inc..
AB A review with 41 refs. **Bispecific** antibodies (BsAb) are one approach to increasing the immunol. effectiveness of therapy with monoclonal antibodies. Preclin. studies have demonstrated that MDX-210 effectively directs type I Fc receptor (Fc.gamma.RI) pos. effector cells to phagocytose tumor cells that overexpress **HER-2**/neu.

L22 ANSWER 17 OF 56 SCISEARCH COPYRIGHT 2002 ISI (R)
2000:184750 The Genuine Article (R) Number: 288CA. Pharmacokinetic-pharmacodynamic relationships of the **bispecific** antibody MDXH210 when combined with interferon gamma (IFNG) in a multiple-dose phase-I study in **HER-2** positive cancer patients.. Lewis L D (Reprint); Cole B F; Fisher J; Waugh M; Wallace P K; Fanger M W; Guyre P; Curnow R; Kaufman P A; Ernstoff M S. MEDAREX INC, ANNANDALE, NJ; DARTMOUTH HITCHCOCK MED CTR, LEBANON, NH 03766. CLINICAL PHARMACOLOGY & THERAPEUTICS (FEB 2000) Vol. 67, No. 2, pp. PI53-PI53. Publisher: MOSBY-YEAR BOOK INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318. ISSN: 0009-9236. Pub. country: USA. Language: English.

L22 ANSWER 18 OF 56 CAPLUS COPYRIGHT 2002 ACS
1999:626072 Document No. 131:256334 Apo-2 ligand-anti-**Her-2** antibody synergism for inducing apoptosis in tumors. Ashkenazi, Avi J.; Phillips, Gail D. (Genentech, Inc., USA). PCT Int. Appl. WO 9948527 A1 19990930, 50 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US6673 19990326. PRIORITY: US 1998-PV79683 19980327.
AB Methods of using synergistically effective amts. of Apo-2 ligand and anti-

Her-2 antibodies to enhance cell death via apoptosis are provided.

L22 ANSWER 19 OF 56 MEDLINE DUPLICATE 9
2000019958 Document Number: 20019958. PubMed ID: 10550549. Production and characterization of mice transgenic for the A and B isoforms of human FcgammaRIII. Amoroso A R; Alpaugh R K; Barth M W; McCall A M; Weiner L M. (Department of Medical Oncology, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111, USA.) CANCER IMMUNOLOGY, IMMUNOTHERAPY, (1999 Nov) 48 (8) 443-55. Journal code: 8605732. ISSN: 0340-7004. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.
AB Fcgamma receptor (FcgammaR) engagement is pivotal for many effector functions of macrophages, polymorphonuclear neutrophils (PMN), and natural killer (NK) cells. Mice transgenic for the A and B isoforms of human (h) FcgammaRIII on macrophages, PMN, and NK cells were constructed to permit the study of mechanisms and potential in vivo strategies to utilize the cytotoxic effector and antigen-presenting functions of cells expressing the hFcgammaR. The present report characterizes the phenotypic and functional expression of hFcgammaRIII in transgenic mice derived by crossing hFcgammaRIIIA and hFcgammaRIIIB transgenic mice. Interleukin-2 (IL-2) induces hFcgammaRIII expression by myeloid cells and their precursors, and these transgenic receptors promote in vitro cytotoxicity and anti-hFcgammaRIII antibody internalization. Splenocytes from untreated and IL-2-treated hFcgammaRIIIA, hFcgammaRIIIB, and hFcgammaRIIIA/B mice exhibited enhanced in vitro cytotoxicity toward **HER-2** /neu-overexpressing SK-OV-3 human ovarian carcinoma cells when incubated with the murine **bispecific** mAb 2B1, which has specificity for **HER-2**/neu and hFcgammaRIII. These results indicate that hFcgammaRIII transgenes are expressed on relevant murine cellular subsets, exhibit inducible up-regulation patterns similar to those seen in humans, and code for functional proteins. hFcgammaRIII transgenic mice exhibiting specific cellular subset expression will permit the examination of strategies designed to enhance hFcgammaRIII-dependent immunological effector functions and will provide a model system in which to evaluate preclinically potential candidate molecules that recognize hFcgammaRIII for the immunotherapy of cancer.

L22 ANSWER 20 OF 56 SCISEARCH COPYRIGHT 2002 ISI (R)
1999:530787 The Genuine Article (R) Number: 212NK. A pilot trial of GM-CSF and MDX-H210 in patients with erbB-2-positive advanced malignancies. Posey J A (Reprint); Raspet R; Verma U; Deo Y M; Keller T; Marshall J L; Hodgson J; Mazumder A; Hawkins M J. UNIV ALABAMA, WALLACE TUMOR INST, 1824 6TH AVE S, BIRMINGHAM, AL 35294 (Reprint); GEORGETOWN UNIV, MED CTR, VINCENT T LOMBARDI CANC RES CTR, DEPT MED, DIV HEMATOL ONCOL, WASHINGTON, DC 20007; MEDAREX INC, ANNANDALE, NJ. JOURNAL OF IMMUNOTHERAPY (JUL 1999) Vol. 22, No. 4, pp. 371-379. Publisher: LIPPINCOTT WILLIAMS & WILKINS. 227 EAST WASHINGTON SQ, PHILADELPHIA, PA 19106. ISSN: 1053-8550. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB MDX-H210 is a chemically, cross-linked, half-humanized **bispecific** antibody composed of F(ab') fragment from monoclonal antibody (mAb) H22 that binds re the high-affinity receptor Fc gamma RI and F(ab') of mAb 520C9 that recognizes the erbB-2 (HER2/neu) oncoprotein. In a previous trial, the murine **bispecific**, MDX-210 at a dose of 7 mg/m(2), was well tolerated and activated monocytes and macrophages in vivo in doses as low as 0.35 mg/m(2). In our multidose trial, granulocyte-macrophage colony-stimulating factor, which increases and activates potential effector cells, was given on days 1-4 at 250 mu g/m(2) s.c. and MDX-H210 was given on day 4 weekly for 4 consecutive weeks. Thirteen patients were treated at dose levels of 1, 3.5, 7, 10, 15, and 20 mg/m(2) without dose-limiting toxicity. Fever, chills, and rigors occurred during and up to 2 h postinfusion and correlated with the time to peak levels of tumor necrosis factor-alpha (median 88.2 pg/ml; range 15.6-887

pg/ml) and interleukin-6 (median 371 pg/ml; range 175-2,149 pg/ml). By the fourth consecutive week of treatment the side effects and cytokine levels decreased significantly. Human antibispecific antibody (HABA) levels were increased by 200- to 500-fold above pretreatment levels in 5 of 11 evaluable patients after 3 weeks of treatment. The monocyte and granulocyte population increased on days 3 and 11 (median 44%; range 18-68% and 42%; 19-71%), respectively, for monocytes and (60%; 43-75% and 74%; 54-82%) on days 4 and 11 for granulocytes. There was a significant decrease in the monocyte populations immediately after MDX-H210 administration (median decrease 73%; range 42-94%) and (52%; 12-72%) on days 4 and 11, respectively. Ten patients completed 4 weeks of treatment. One patient had a 48% reduction in an index lesions and six patients had stable disease at the time of evaluation. Three patients progressed before the fourth week. The therapy was generally well tolerated with toxicity, primarily, limited to the days of treatment.

L22 ANSWER 21 OF 56 MEDLINE DUPLICATE 10
1999295740 Document Number: 99295740. PubMed ID: 10369066. Antibody dependent cellular phagocytosis (ADCP) and antibody dependent cellular cytotoxicity (ADCC) of breast cancer cells mediated by **bispecific** antibody, MDX-210. Watanabe M; Wallace P K; Keler T; Deo Y M; Akewanlop C; Hayes D F. (The Breast Cancer Program, Lombardi Cancer Center, Georgetown University Medical Center, Washington, DC 20007, USA.) BREAST CANCER RESEARCH AND TREATMENT, (1999 Feb) 53 (3) 199-207. Journal code: 8111104. ISSN: 0167-6806. Pub. country: Netherlands. Language: English.
AB BACKGROUND: MDX-210 is a **bispecific** antibody (BsAb) with specificity for both the proto-oncogene product of **HER-2** /neu (c-erbB-2) and FcgammaRI (CD64). **HER-2**/neu is overexpressed in malignant tissue of approximately 30% of patients with breast cancer, and FcgammaRI is expressed on human monocytes, macrophages, and IFN-gamma activated granulocytes. We investigated phagocytosis and cytolysis of cultured human breast cancer cells by human monocyte-derived macrophages (MDM) mediated by BsAb MDX-210, its partially humanized derivative (MDX-H210), and its parent MoAb 520C9 (anti-**HER-2**/neu) under various conditions. MATERIALS AND METHODS: Purified monocytes were cultured with GM-CSF, M-CSF, or no cytokine for five or six days. Antibody dependent cellular phagocytosis (ADCP) and cytolysis (ADCC) assays were performed with the MDM and **HER-2**/neu positive target cells (SK-BR-3). ADCP was measured by two-color fluorescence flow cytometry using PKH2 (green fluorescent dye) and phycoerythrin-conjugated (red) monoclonal antibodies (MoAb) against human CD14 and CD11b. ADCC was measured with a non-radioactive LDH detection kit. RESULTS: Both BsAb MDX-210 (via FcgammaRI) and MoAb 520C9 (mouse IgG1, via FcgammaRII) mediated similar levels of ADCP and ADCC. ADCP mediated by BsAb MDX-H210 was identical to that mediated by BsAb MDX-210. Confocal microscopy demonstrated that dual-labeled cells represented true phagocytosis. Both ADCP and ADCC were higher when MDM were pre-incubated with GM-CSF than when incubated with M-CSF. CONCLUSIONS: BsAb MDX-210 is as active in vitro as the parent MoAb 520C9 in inducing both phagocytosis and cytolysis of MDM. MDX-210 and its partially humanized derivative, MDX-H210, mediated similar levels of ADCP. GM-CSF appears to superior to M-CSF in inducing MDM-mediated ADCC and ADCP. These studies support the ongoing clinical investigations of BsAb MDX-210 and its partially humanized derivative.

L22 ANSWER 22 OF 56 SCISEARCH COPYRIGHT 2002 ISI (R)
1999:848642 The Genuine Article (R) Number: 251GE. **Bispecific** antibody MDX-H210 (Fc gamma RI x **HER-2**/neu) in combination with G-CSF: clinical and biological phase I results. Valerius T (Reprint); Repp R; Wieland G; Stockmeyer B; Deo Y M; vanOijk H; Kalden J R; vandeWinkel J G J; Gramatzki M. UNIV ERLANGEN NURNBERG, DEPT MED 3, NURNBERG, GERMANY; UNIV ERLANGEN NURNBERG, DEPT GYNECOL, NURNBERG, GERMANY; UNIV UTRECHT HOSP, DEPT IMMUNOL, UTRECHT, NETHERLANDS; MEDAREX

INC, ANNANDALE, NJ. EUROPEAN JOURNAL OF CANCER (OCT 1999) Vol. 35, Supp. [5], pp. 92-92. Publisher: PERGAMON-ELSEVIER SCIENCE LTD. THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND. ISSN: 0959-8049. Pub. country: GERMANY; NETHERLANDS; USA. Language: English.

L22 ANSWER 23 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2000:19428 Document No.: PREV200000019428. **Bispecific** antibody MDX-H210 (FcgammaRI X **HER-2**/neu) in combination with G-CSF: Clinical and biological phase I results. Valerius, T. (1); Repp, R.; Wieland, G.; Stockmeyer, B.; Deo, Y. M.; van Oijk, H.; Kalden, J. R.; van de Winkel, J. G. J.; Gramatzki, M.. (1) Department of Medicine III, University of Erlangen, Nuernberg Germany. European Journal of Cancer, (Oct., 1999) Vol. 35, No. SUPPL. 5, pp. S50. Meeting Info.: 5th International Symposium on the Biological Therapy of Cancer: From Basic Research to Clinical Applications Munich, Germany October 27-30, 1999 Biological Therapeutics Development Group of the European Organisation for Research and Treatment of Cancer. ISSN: 0959-8049. Language: English.

L22 ANSWER 24 OF 56 SCISEARCH COPYRIGHT 2002 ISI (R)
1999:340393 The Genuine Article (R) Number: 190AX. A phase I study of a HER2/neu **bispecific** antibody with granulocyte-colony-stimulating factor in patients with metastatic breast cancer that overexpresses HER2/neu. Pullarkat V; Deo Y; Link J; Spears L; Marty V; Curnow R; Groshen S; Gee C; Weber J S (Reprint). USC NORRIS COMPREHENS CANC CTR, 1441 EASTLAKE AVE, LOS ANGELES, CA 90049 (Reprint); USC NORRIS COMPREHENS CANC CTR, LOS ANGELES, CA 90049; MEDAREX INC, ANNANDALE, NJ. CANCER IMMUNOLOGY IMMUNOTHERAPY (APR 1999) Vol. 48, No. 1, pp. 9-21. Publisher: SPRINGER VERLAG. 175 FIFTH AVE, NEW YORK, NY 10010. ISSN: 0340-7004. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB A phase I study of escalating doses of humanized **bispecific** antibody (bsAb) MDX-H210 with granulocyte-colony-stimulating factor (G-CSF) was conducted in patients with metastatic breast cancer that overexpressed HER2/neu. The main objectives of the study were to define the maximal tolerated dose (MTD) of MDX-H210 when combined with G-CSF, to measure the pharmacokinetics of MDX-H210 when administered with G-CSF, and to determine the toxicity, biological effects and possible therapeutic effect of MDX-H210 with G-CSF. MDX-H210 is a F(ab)' x F(ab)' humanized **bispecific** murine antibody that binds to both HER2/neu and the Fc gamma R1 receptor (CD64), and was administered intravenously weekly for three doses followed by a 2-week break and then three more weekly doses. A total of 23 patients were treated, and doses were escalated from 1 mg/m(2) to 40 mg/m(2) with no MTD reached. The toxicity of the bsAb + G-CSF combination was modest, with no dose-limiting toxicity noted: 19 patients had fevers, 7 patients had diarrhea, and 3 patients had allergic reactions that did not limit therapy. The beta-elimination half-life varied from 4 h to 8 h at doses up to 20 mg/m(2) Significant release of cytokines interleukin-6, G-CSF, and tumor necrosis factor cl was observed after administration of bsAb. Circulating monocytes disappeared within I h of bsAb infusion, which correlated with binding of bsAb, noted by flow-cytometric analysis. Significant levels of human anti-(**bispecific** antibody) were measured in the plasma of most patients by the third infusion. No objective clinical responses were seen in this group of heavily pre-treated patients.

L22 ANSWER 25 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1998:467429 Document No.: PREV199800467429. Combining anti-CD3 activated T cells armed with anti-CD3xanti-Her2 **bispecific** antibody may provide an additional anti-tumor effect after stem cell transplant for breast cancer. Sen, M.; Wankowski, D. M.; Lum, L. G.. St. Luke's Med. Cent., Milwaukee, WI USA. Experimental Hematology (Charlottesville), (Aug., 1998) Vol. 26, No. 8, pp. 743. Meeting Info.: 27th Annual Meeting of the International Society for Experimental Hematology Vancouver,

British Columbia, Canada August 1-5, 1998 International Society for Experimental Hematology. ISSN: 0301-472X. Language: English.

L22 ANSWER 26 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1998:459651 Document No.: PREV199800459651. Phase II trial of the **bispecific** antibody MDX-H210 (anti-Her2/Neu X anti-CD64) combined with GM-CSF in patients with advanced prostate and renal cell carcinoma that express Her2/neu. James, N. (1); Atherton, P. (1); Koletsky, A.; Tchekmedyian, N.; Curnow, R.. (1) CRC Inst. Cancer Studies, Birmingham B15 2TH UK. British Journal of Cancer, (1998) Vol. 78, No. SUPPL. 2, pp. 19. Meeting Info.: Joint Meeting of the British Oncological Association, the Association of Cancer Physicians and the Royal College of Radiologists Nottingham, England, UK July 5-7, 1998 Association of Cancer Physicians. ISSN: 0007-0920. Language: English.

L22 ANSWER 27 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1999:103134 Document No.: PREV199900103134. High doses of **bispecific** antibody MDXH210 (FcyRI x **HER-2**/NEU) in combination with G-CSF can be administered to patients with metastatic breast cancer. Valerius, Th.; Repp, R.; Wieland, G.; Deo, Y.; Van Oijk, H.; Van De Winkel, J. G.; Kalden, J. R.; Lang, N.; Gramatzki, M.. Dep. Med. III Gynecology, Univ. Erlangen, Erlangen Germany. Annals of Hematology, (1998) Vol. 77, No. SUPPL. 2, pp. S13. Meeting Info.: Annual Congress of the German and Austrian Societies of Hematology and Oncology Frankfurt, Germany October 25-28, 1998 Austrian Society of Hematology and Oncology. ISSN: 0939-5555. Language: English.

L22 ANSWER 28 OF 56 MEDLINE DUPLICATE 11
1998208286 Document Number: 98208286. PubMed ID: 9548506. Generation of **HER-2**/neu-specific cytotoxic neutrophils in vivo: efficient arming of neutrophils by combined administration of granulocyte colony-stimulating factor and Fcgamma receptor I **bispecific** antibodies. Heijnen I A; Rijks L J; Schiel A; Stockmeyer B; van Ojik H H; Dechant M; Valerius T; Keler T; Tutt A L; Glennie M J; van Royen E A; Capel P J; van de Winkel J G. (Department of Immunology, University Hospital Utrecht, The Netherlands.) JOURNAL OF IMMUNOLOGY, (1997 Dec 1) 159 (11) 5629-39. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Abs are able to induce inflammatory antitumor responses by recruiting IgG Fc receptor (FcgammaR)-bearing cytotoxic effector cells. We recently described the capacity of the high affinity FcgammaRI (CD64) to trigger cytotoxic activity of neutrophils (PMN) during granulocyte CSF (G-CSF) treatment. To take advantage of FcgammaRI as a cytotoxic trigger molecule on PMN, two Ab constructs were prepared. We show that a chimeric human IgG1 Ab (Ch520C9) and an anti-FcgammaRI **bispecific** Ab (BsAb; 22x520C9), both directed to the proto-oncogene product **HER-2**/neu, interact with FcgammaRI. In addition, both Ab constructs mediate enhanced lysis of **HER-2**/neu-expressing tumor cells by G-CSF-primed PMN. However, engagement of FcgammaRI by Ch520C9 was inhibited by human serum IgG, thereby abrogating the enhanced Ch520C9-mediated cytotoxicity. BsAb 22x520C9, which binds FcgammaRI outside the ligand binding domain, effectively recruits the cytotoxic potential of FcgammaRI on G-CSF-primed PMN regardless of the presence of human serum. These results indicate that under physiologic conditions, serum IgG impairs activation of FcgammaRI-mediated cytotoxicity by conventional antitumor Abs. The IgG blockade can be circumvented with anti-FcgammaRI BsAbs. Using human FcgammaRI transgenic mice we demonstrate that BsAb 22x520C9 is able to engage FcgammaRI in vivo. BsAb 22x520C9 injected i.v. was readily detected on circulating PMN of G-CSF-treated transgenic animals. In addition, we showed that PMN remain "armed" with BsAb 22x520C9 during migration to inflammatory sites, and that after isolation such PMN specifically lyse **HER-2**/neu-expressing tumor cells. These results point to the possibility of

targeting anti-FcgammaRI BsAbs to G-CSF-primed PMN in vivo, endowing them with specific anti-tumor activity.

L22 ANSWER 29 OF 56 MEDLINE DUPLICATE 12
1998043637 Document Number: 98043637. PubMed ID: 9373259. FcalphaRI (CD89) as a novel trigger molecule for **bispecific** antibody therapy. Valerius T; Stockmeyer B; van Spriel A B; Graziano R F; van den Herik-Oudijk I E; Repp R; Deo Y M; Lund J; Kalden J R; Gramatzki M; van de Winkel J G. (Department of Medicine III, University of Erlangen, Nurnberg, Germany.) BLOOD, (1997 Dec 1) 90 (11) 4485-92. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Promising results from clinical trials with unconjugated antibodies stimulated renewed interest in immune effector mechanisms of monoclonal antibodies (MoAbs). We investigated the potential of IgA as antibody isotype for cell- or complement-mediated tumor cell lysis and assessed the potential of its myeloid Fc receptor, FcalphaRI (CD89), as trigger molecule for **bispecific** antibody (BsAb)-mediated immunotherapy. Comparing hapten-directed antibodies of human IgA2 with IgG1 or IgG3 isotypes, we found all three to mediate effective killing of sensitized tumor target cells in whole blood assays. Analysis of effector mechanisms showed IgG-mediated lysis to be predominantly complement-dependent, whereas IgA-dependent killing was primarily effector cell-mediated. A comparison of effector cell populations in antibody-dependent cell-mediated cytotoxicity (ADCC) showed neutrophils to be most important for IgA-dependent tumor cell killing, involving FcalphaRI as shown with Fc receptor blocking antibodies. Reverse ADCC experiments against target cells sensitized with Fc receptor antibodies, or assays with FcalphaRI-directed **bispecific** antibodies confirmed FcalphaRI as effective trigger molecule in polymorphonuclear neutrophil (PMN)-mediated lysis. During granulocyte colony-stimulating factor (G-CSF) therapy, (FcalphaRI x **HER-2**/neu) **bispecific** antibodies induced enhanced killing of **HER-2**/neu positive SK-BR-3 breast cancer cells in whole blood assays. This enhanced cytotoxicity was paralleled by increased PMN counts, which lead to higher effector to target cell ratios in G-CSF-primed blood. Furthermore, **bispecific** antibodies, directed to FcalphaRI and Candida albicans, enhanced neutrophils' phagocytosis of fungi. In summary, these results identify IgA as an effective antibody isotype for immunotherapy, working primarily via FcalphaRI on neutrophils. They suggest FcalphaRI-directed **bispecific** antibodies and G-CSF to be an attractive combination for malignant or infectious diseases.

L22 ANSWER 30 OF 56 SCISEARCH COPYRIGHT 2002 ISI (R)
97:694383 The Genuine Article (R) Number: XV559. **Bispecific** antibody-dependent cellular cytotoxicity of HER2/neu-overexpressing tumor cells by Fc gamma receptor type I-expressing effector cells. Keler T; Graziano R F; Mandal A; Wallace P K; Fisher J; Guyre P M; Fanger M W; Deo Y M (Reprint). MEDAREX INC, 1545 ROUTE 22 E, ANNANDALE, NJ 08801 (Reprint); MEDAREX INC, ANNANDALE, NJ 08801; DARTMOUTH COLL SCH MED, DEPT MICROBIOL, LEBANON, NH 03756; DARTMOUTH COLL SCH MED, DEPT PHYSIOL, LEBANON, NH 03756; DARTMOUTH COLL SCH MED, DEPT MED, LEBANON, NH 03756. CANCER RESEARCH (15 SEP 1997) Vol. 57, No. 18, pp. 4008-4014. Publisher: AMER ASSOC CANCER RESEARCH. PUBLIC LEDGER BLDG, SUITE 816, 150 S. INDEPENDENCE MALL W., PHILADELPHIA, PA 19106. ISSN: 0008-5472. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A **bispecific** antibody, MDX-H210, was developed to target cytotoxic effector cells expressing Fc gamma receptor type I(Fc gamma RI, CD64) to HER2/neu-overexpressing tumor cells, HER2/neu is an appropriate target for immunotherapy due to the high level of expression of this proto-oncogene in a variety of malignancies, The expression of Fc gamma RI is limited primarily to cytotoxic immune cells, including monocytes, macrophages, and cytokine-activated polymorphonuclear (PMN) cells,

Therefore, tumor cells bound with MDX-H210 can be selectively recognized by effector cells with cytotoxic potential, MDX-H210 was prepared by chemical conjugation of Fab' fragments derived from the HER2/neu-specific monoclonal antibody, 520C9, and the Fc gamma RI-specific monoclonal antibody, H22, This **bispecific** molecule demonstrated specific, dose-dependent, and saturable binding to both HER2/neu- and Fc gamma RI-expressing cells, A solid-phase immunoassay that demonstrated simultaneous and specific binding to both antigens was used to confirm the **bispecific** nature of MDX-H210. Monocytes and PMN cells mediated MDX-H210-dependent lysis of HER2/neu-overexpressing cell lines derived from breast, ovarian, and lung carcinomas. IFN-gamma treatment of monocytes enhanced antibody-dependent cellular cytotoxicity, whereas IFN-gamma and granulocyte colony-stimulating factor were required for PILIN cell-mediated tumor cell lysis. In addition, MDX-H210 elicited tumor necrosis factor-alpha secretion from monocytes when cultured in the presence of HER2/neu-positive target cells, These in vitro data suggest that targeting tumor cells to Fc gamma RI with MDX-H210 may be an effective treatment for malignancies that overexpress HER2/neu. The in vivo cytotoxic potential of MDX-H210 may be enhanced by combination therapy with the cytokines granulocyte colony-stimulating factor and IFN-gamma, which up-regulate Fc gamma RI expression on cytotoxic effector cells.

L22 ANSWER 31 OF 56 MEDLINE DUPLICATE 13
97178947 Document Number: 97178947. PubMed ID: 9044847. Preclinical studies with Fc(gamma)R **bispecific** antibodies and granulocyte colony-stimulating factor-primed neutrophils as effector cells against **HER-2**/neu overexpressing breast cancer. Stockmeyer B; Valerius T; Repp R; Heijnen I A; Buhring H J; Deo Y M; Kalden J R; Gramatzki M; van de Winkel J G. (Department of Medicine III, University of Erlangen-Nurnberg, Germany.) CANCER RESEARCH, (1997 Feb 15) 57 (4) 696-701. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Immunotherapies directed to the proto-oncogene product **HER-2**/neu, which is overexpressed on a subset of breast and other carcinomas, currently receive considerable attention. We have investigated cell-mediated effector mechanisms of **HER-2**/neu antibodies against breast cancer cell lines. Compared to unfractionated control blood, whole blood from patients during granulocyte colony-stimulating factor (G-CSF) treatment exhibits significantly enhanced lysis ($P < 0.001$) of SK-BR-3 cells in the presence of **HER-2**/neu antibody 520C9. The extent of tumor cell killing correlated positively ($r = 0.74$) to polymorphonuclear neutrophil (PMN) blood counts. Fractionation of whole blood into plasma, mononuclear cells, and PMNs showed major killing capacity to reside in the granulocyte fraction. PMNs were efficiently cytolytic with a panel of **HER-2**/neu antibodies and against various breast cancer cell lines. Experiments with blocking antibodies to Fc(gamma)R documented Fc(gamma)RII (CD32) as the major trigger molecule for monoclonal antibody 502C9-mediated cytotoxicity. Killing via 520C9 was significantly influenced by an allotypic polymorphism of Fc(gamma)RIIa, the CD32 molecule expressed on PMNs. In reverse antibody-dependent cell-mediated cytotoxicity experiments with a panel of **HER-2**/neu-directed **bispecific** antibodies, Fc(gamma)RIII (CD16) proved to be an efficient trigger molecule in blood from healthy volunteers. During G-CSF treatment, however, Fc(gamma)RI (CD64)-expressed on monocytes and G-CSF primed, but not on healthy donor PMNs-became the predominant cytotoxic trigger molecule. Thus, G-CSF application increased effector cell numbers for **HER-2**/neu-directed immunotherapy, and G-CSF primed PMNs proved particularly effective with a [**HER-2**/neu x Fc(gamma)RI] **bispecific** antibody. These findings support clinical trials with **HER-2**/neu-directed antibodies in combination with G-CSF in breast cancer patients

overexpressing **HER-2**/neu.

L22 ANSWER 32 OF 56 SCISEARCH COPYRIGHT 2002 ISI (R)
97:825969 The Genuine Article (R) Number: XX830. **Bispecific** antibody MDX210 (Fc gamma RI x **HER-2**/neu) in combination with G-CSF: Results of a phase I trial in patients with metastatic breast cancer. Valerius T (Reprint); Stockmeyer B; Repp R; Deo Y; vanOijk H; vandeWinkel J G; Kalden J R; Gramatzki M. UNIV ERLANGEN NURNBERG, DEPT MED, D-8520 ERLANGEN, GERMANY; UNIV UTRECHT HOSP, DEPT IMMUNOL, UTRECHT, NETHERLANDS; UNIV UTRECHT HOSP, DEPT ONCOL, UTRECHT, NETHERLANDS; MEDAREX INC, ANNANDALE, NJ. EUROPEAN JOURNAL OF CANCER (SEP 1997) Vol. 33, Supp. [8], pp. 676-676. Publisher: PERGAMON-ELSEVIER SCIENCE LTD. THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD, ENGLAND OX5 1GB. ISSN: 0959-8049. Pub. country: GERMANY; NETHERLANDS; USA. Language: English.

L22 ANSWER 33 OF 56 MEDLINE DUPLICATE 14
97390704 Document Number: 97390704. PubMed ID: 9247561. Induction of multiple anti-c-erbB-2 specificities accompanies a classical idiotypic cascade following 2B1 **bispecific** monoclonal antibody treatment. Clark J I; Alpaugh R K; von Mehren M; Schultz J; Gralow J R; Cheever M A; Ring D B; Weiner L M. (Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA 19111, USA.) CANCER IMMUNOLOGY, IMMUNOTHERAPY, (1997 Jul) 44 (5) 265-72. Journal code: 8605732. ISSN: 0340-7004. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.
AB The **bispecific** monoclonal antibody (bsmAb) 2B1, targeting the extracellular domain of c-erbB-2, the protein product of the **HER-2**/neu proto-ocogene, and Fc gamma RIII (CD16), expressed by human natural killer cells, neutrophils and differentiated monocytes, mediates the specific cytotoxic activity of these effector cells to tumor cells. A group of 24 patients with c-erbB-2-overexpressing tumors were treated with intravenously administered 2B1 in a phase I clinical trial and followed after treatment to evaluate the diversity and extent of the 2B1-induced humoral immune responses. As expected, 17 of 24 patients developed human anti-(murine Ig) antibodies (HAMA) to whole 2B1 IgG in a range from 100 ng/ml to more than 50000 ng/ml; 10 of these patients (42%) had strong (at least 1000 ng/ml) HAMA responses, some of which were still detectable at day 191. These responses were usually associated with similar reactivity to the F(ab')2 fragments of the parental antibodies 520C9 (anti-c-erbB-2) and 3G8 (anti-CD16). We sought evidence of an idiotypic cascade induction, indicating a prolonged specific treatment-induced effect on at least one selected target of 2B1. Using competition-based enzyme-linked immunosorbent assays, specific anti-idiotypic antibodies (Ab2) were detectable against 520C9 in 11 patients and against 3G8 in 13 patients. Peak anti-idiotypic antibodies generally occurred 3-5 weeks from treatment initiation, with a downward trend thereafter. There was a statistically significant correlation among the induction of significant HAMA responses, anti-idiotypic antibody production and the development of antibodies to c-erbB-2. The anti-c-erbB-2 responses, which were distinct from anti-anti-idiotypic (Ab3) antibodies, were detected in the post-treatment sera of 6/16 patients examined. No obvious correlation could be made between the development of humoral immune responses, the dose received, and the clinical response. Future investigation involving 2B1 therapy will concentrate on investigating an association of these humoral responses to any c-erbB-2-specific cellular responses. Manipulations of 2B1 therapy effects that augment immunity to c-erbB-2 could provide additional avenues for immunotherapy with this and other **bispecific** antibodies.

L22 ANSWER 34 OF 56 MEDLINE DUPLICATE 15
1998098164 Document Number: 98098164. PubMed ID: 9435875. Clinical evaluation of the **bispecific** antibody MDX-H210 (anti-Fc gamma RI x anti-**HER-2**/neu) in combination with

granulocyte-colony-stimulating factor (filgrastim) for treatment of advanced breast cancer. van Ojik H H; Repp R; Groenewegen G; Valerius T; van de Winkel J G. (Department of Internal Medicine, University Hospital, Utrecht, The Netherlands.) CANCER IMMUNOLOGY, IMMUNOTHERAPY, (1997 Nov-Dec) 45 (3-4) 207-9. Journal code: 8605732. ISSN: 0340-7004. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

L22 ANSWER 35 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1997:103242 Document No.: PREV199799402445. Antibody dependent cellular phagocytosis (ADCP) against **HER-2**/neu positive human breast cancer cells mediated by **bispecific** monoclonal antibody (BsAb) MDX-210. Watanabe, M. (1); Wallace, P.; Graziano, R.; Deo, Y.; Kufe, D. W.; Hayes, D. F.. (1) Dana-Farber Cancer Inst., Boston, MA 02115 USA. Breast Cancer Research and Treatment, (1996) Vol. 41, No. 3, pp. 247. Meeting Info.: 19th Annual San Antonio Breast Cancer Symposium on Breast Cancer Research and Treatment San Antonio, Texas, USA December 11-14, 1996 ISSN: 0167-6806. Language: English.

L22 ANSWER 36 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1997:103241 Document No.: PREV199799402444. **Bispecific** antibody (BsAb) 520C9 X 22 (MDX-210) and interferon gamma (IFN-gamma) is an immunologically active treatment for patients with metastatic adenocarcinomas that overexpress **HER-2**/neu (**HER-2**. Kaufman, P. A. (1); Lewis, L. D.; Barth, R. J.; Guyre, P. M.; Wallace, P. K.; Memoli, V. A.; Wells, W.; Deo, Y. M.; Valone, F. H.; Fisher, J.; Waugh, M.; Mackay, K.; Mrozek-Orlowski, M.; Phipps, K.; Fanger, M. W.; Ernstoff, M. S.. (1) Comprehensive Breast Care Program, Norris Cotton Cancer Cent., Dartmouth-Hitchcock Med. Cent., Lebanon, NH 03756 USA. Breast Cancer Research and Treatment, (1996) Vol. 41, No. 3, pp. 246. Meeting Info.: 19th Annual San Antonio Breast Cancer Symposium on Breast Cancer Research and Treatment San Antonio, Texas, USA December 11-14, 1996 ISSN: 0167-6806. Language: English.

L22 ANSWER 37 OF 56 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 16
96157946 EMBASE Document No.: 1996157946. Targeting growth factor receptors with **bispecific** molecules. Mokotoff M.; Chen J.; Zhou J.-H.; Ball E.D.. School of Pharmacy, Dept. of Pharmaceutical Sciences, University of Pittsburgh,Pittsburgh, PA 15261, United States. Current Medicinal Chemistry 3/2 (87-100) 1996.
ISSN: 0929-8673. CODEN: CMCHE7. Pub. Country: Netherlands. Language: English. Summary Language: English.
AB Peptide growth factor receptors on the surface of malignant cells bind to their ligands with high affinity, resulting in intracellular responses which cause differentiation, growth, and the survival of these cells. Peptide growth factors, or monoclonal antibodies (mAbs) which target growth factor receptors, have been conjugated to drugs, toxins, radionuclides, or other mAbs that recognize/activate effector cells which can phagocytose or kill. These types of conjugated products, which have the ability to kill malignant cells, we call **bispecific** molecules (BsMol) and is the basis of this review article. The growth factors/receptors covered include .alpha. - and .beta.-melanocyte stimulating hormone (MSH), bombesin/gastrin releasing peptide (BN/GRP), epidermal growth factor (EGF), **HER-2**/neu oncogene protein (p185(HER2), interleukin-2, and somatostatin. The preparation and biological use/activity of the following BsMol are discussed: .beta.-MSH-daunomycin, [Nle4, D-Phe7]MSH-anti-CD3, 111In-DTPA-bis-.alpha.-MSH, DAB389-MSH, mAb22-Lys-BN, mAb22-Antag1, anti-EGFR/anti-CD3, DOXER2, DAB389EGF, 111In-DTPA-225, anti-p185(HER2)-SAP, 2B1, MDX-210, humAb4D5-8 x humAbUCHT1, DAB486IL-2, 111In-DTPA-octreotide (OctreoScan.RTM.), OX-26-NGF, and IVA039.1.

L22 ANSWER 38 OF 56 CAPLUS COPYRIGHT 2002 ACS
1996:380457 Document No. 125:48653 **HER-2**/neu targeted

immunotherapy: A pilot study of multi-dose MDX-210 in patients with breast or ovarian cancers that overexpress **HER-2**/neu and a report of an increased incidence of **HER-2**/neu overexpression in metastatic breast cancer. Kaufman, P. A.; Guyre, P. M.; Lewis, L. D.; Valone, F. H.; Memoli, V.; Wells, W. A. A.; Deo, Y. M.; Ernstoff, M. S.; Fisher, J.; et al. (Department Medicine, Norris Cotton Cancer Center, Lebanon, NH, 03756, USA). Tumor Targeting, 2(1), 17-28 (English) 1996. CODEN: TUTAF9. ISSN: 1351-8488. Publisher: Chapman & Hall.

AB MDX-210 is an immunol. active **bispecific** antibody (BsAb) which in preclin. studies targets Fc.gamma.RI-expressing effector cells to kill tumor cells that overexpress **HER-2**/neu. The authors now report the authors' findings of a phase I clin. trial of multiple doses of MDX-210 in patients with metastatic breast or ovarian cancers overexpressing **HER-2**/neu. The primary goals of this pilot clin. trial were to evaluate the clin. toxicity and immunol. activity of multiple infusions of MDX-210. Therapy with MDX-210 resulted in acute monocytopenia, which was expected, given the constitutive expression of Fc.gamma.RI by circulating monocytes. No significant hematol. toxicity was noted, and clin. toxicity was mild. Increased plasma levels of tumor necrosis factor .alpha. (TNF-.alpha.), interleukin 6 (IL-6) and granulocyte colony-stimulating factor (G-CSF) were noted subsequent to therapy with MDX-210. These findings suggest that sequential dosing of MDX-210 is assocd. with minimal toxicity, and that MDX-210 is immunol. active. Of note, treatment with successive doses of MDX-210 appears to lead to desensitization with regard to the development of monocytopenia, as well as the plasma increases in TNF-.alpha., IL-6, and G-CSF. Furthermore, some patients have shown evidence of active antitumor immunity following therapy with MDX-210. Addnl., the authors now report the authors' findings on the overall incidence of **HER-2**/neu overexpression in metastatic breast and advanced ovarian cancer that the authors have found while screening patients for several BsAb trials. In this population, the incidence of **HER-2**/neu overexpression is 72% in patients with metastatic breast cancer, and 53% in patients with advanced ovarian cancer.

L22 ANSWER 39 OF 56 MEDLINE DUPLICATE 17

95395483 Document Number: 95395483. PubMed ID: 7545221. Phase Ia/Ib trial of **bispecific** antibody MDX-210 in patients with advanced breast or ovarian cancer that overexpresses the proto-oncogene **HER-2**/neu. Valone F H; Kaufman P A; Guyre P M; Lewis L D; Memoli V; Deo Y; Graziano R; Fisher J L; Meyer L; Mrozek-Orlowski M; +. (Department of Medicine, Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA.) JOURNAL OF CLINICAL ONCOLOGY, (1995 Sep) 13 (9) 2281-92. Journal code: 8309333. ISSN: 0732-183X. Pub. country: United States. Language: English.

AB PURPOSE: MDX-210 is a **bispecific** antibody that binds simultaneously to type I Fc receptors for immunoglobulin G (IgG) (Fc gamma RI) and to the **HER-2**/neu oncogene protein product. MDX-210 effectively directs Fc gamma RI-positive effector cells such as monocytes and macrophages to phagocytose or kill tumor cells that overexpress **HER-2**/neu. The goals of this phase Ia/Ib trial were to determine the maximum-tolerated dose (MTD) and/or the optimal biologic dose (OBD) of MDX-210. PATIENTS AND METHODS: Patients with advanced breast or ovarian cancer that overexpressed **HER-2**/neu were eligible for treatment. Cohorts of three patients received a single intravenous (IV) infusion of MDX-210 at increasing dose levels from 0.35 to 10.0 mg/m2. RESULTS: Treatment was well tolerated, with most patients experiencing transient grade 1 to 2 fevers, malaise, and hypotension only. Two patients experienced transient grade 3 hypotension at 10.0 mg/m2. Transient monocytopenia and lymphopenia developed at 1 to 2 hours, but no other hematologic changes were observed. Doses of MDX-210 > or = 3.5 mg/m2 saturated > or = 80% of monocyte Fc gamma RI and produced peak plasma concentrations > or = 1 microgram/mL,

which is greater than the concentration for optimal monocyte/macrophage activation in vitro. Elevated plasma levels of the monocyte products tumor necrosis factor alpha (TNF alpha), interleukin-6 (IL-6), granulocyte colony-stimulating factor (G-CSF), and neopterin were observed with maximal levels at doses > or = 7.0 mg/m2. Localization of MDX-210 in tumor tissue was demonstrated in two patients. One partial and one mixed tumor response were observed among 10 assessable patients. CONCLUSION: MDX-210 is immunologically active at well-tolerated doses. The MTD and OBD is 7 to 10 mg/m2.

L22 ANSWER 40 OF 56 SCISEARCH COPYRIGHT 2002 ISI (R)
96:18396 The Genuine Article (R) Number: TH910. PHASE-I TRIAL OF MDX210 (**BISPECIFIC** ANTIBODY FC-GAMMA-RI X **HER-2**/NEU) IN COMBINATION WITH G-CSF IN PATIENTS WITH BREAST-CANCER. REPP R (Reprint); VALERIUS T; WIELAND G; OETZEL C; DEO Y; VANDEWINKEL J G; KALDEN J R; LANG N; GRAMATZKI M. UNIV ERLANGEN NURNBERG, DEPT MED 3, W-8520 ERLANGEN, GERMANY; UNIV ERLANGEN NURNBERG, DEPT GYNECOL, W-8520 ERLANGEN, GERMANY; UNIV UTRECHT HOSP, DEPT IMMUNOL, UTRECHT, NETHERLANDS; MEDAREX INC, ANNANDALE, NJ, 00000. BLOOD (15 NOV 1995) Vol. 86, No. 10, Supp. 1, pp. 2017. ISSN: 0006-4971. Pub. country: GERMANY; NETHERLANDS; USA. Language: ENGLISH.

L22 ANSWER 41 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1996:49712 Document No.: PREV199698621847. Phase I trial of MDX210 (**bispecific** antibody Fc-gamma-RI x **HER-2**/neu) in combination with G-CSF in patients with breast cancer. Repp, R. (1); Valerius, T.; Wieland, G.; Oetzel, C.; Deo, Y.; Van De Winkel, J. G.; Kalden, J. R.; Lang, N.; Gramatzki, M.. (1) Dep. Med. III, Univ. Erlangen-Nuernberg, Erlangen Germany. Blood, (1995) Vol. 86, No. 10 SUPPL. 1, pp. 507A. Meeting Info.: 37th Annual Meeting of the American Society of Hematology Seattle, Washington, USA December 1-5, 1995 ISSN: 0006-4971. Language: English.

L22 ANSWER 42 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1995:187158 Document No.: PREV199598201458. Schedule dependent immunological stimulation by **bispecific** antibody (BsAb) MDX-210 (anti-Fc-gamma-RI x anti-**HER-2**/neu) in patients with breast or ovarian cancers that over express **HER-2**/neu. Valone, F. H.; Kaufman, P. A.; Gericke, G.; Fisher, J.; Guyre, P. M.; Memoli, V.; Barth, R.; Phipps, K.; Lewis, L.; Deo, Y.; Fanger, M. W.. Dartmouth-Hitchcock Med. Cent., Lebanon, NH USA. Proceedings of the American Association for Cancer Research Annual Meeting, (1995) Vol. 36, No. 0, pp. 500. Meeting Info.: Eighty-sixth Annual Meeting of the American Association for Cancer Research Toronto, Ontario, Canada March 18-22, 1995 ISSN: 0197-016X. Language: English.

L22 ANSWER 43 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1995:187130 Document No.: PREV199598201430. Functional analysis of anti-**HER-2**/anti CD3 bsmAb on breast cancer tumor clones. Nistico, P. (1); Del Bello, D. (1); Digiesi, G. (1); Vola, R.; Fraioli, R. (1); Venturo, I. (1); Malavasi, F.; Natali, P. G. (1). (1) Regina Elena Cancer Inst. Rome, Rome Italy. Proceedings of the American Association for Cancer Research Annual Meeting, (1995) Vol. 36, No. 0, pp. 495. Meeting Info.: Eighty-sixth Annual Meeting of the American Association for Cancer Research Toronto, Ontario, Canada March 18-22, 1995 ISSN: 0197-016X. Language: English.

L22 ANSWER 44 OF 56 MEDLINE DUPLICATE 18
96129485 Document Number: 96129485. PubMed ID: 8581387. Clinical trials of **bispecific** antibody MDX-210 in women with advanced breast or ovarian cancer that overexpresses **HER-2**/neu. Valone F H; Kaufman P A; Guyre P M; Lewis L D; Memoli V; Ernstoff M S; Wells W; Barth R; Deo Y; Fisher J; +. (Norris Cotton Cancer Center, Lebanon, NH,

USA.) JOURNAL OF HEMATOTHERAPY, (1995 Oct) 4 (5) 471-5. Journal code: 9306048. ISSN: 1061-6128. Pub. country: United States. Language: English.

AB MDX-210 is a **bispecific** antibody (BsAb) that recognizes Fc gamma R1 on monocytes and macrophages and the cell surface product of the **HER-2**/neu oncogene, which is overexpressed on some breast and ovarian cancers. Clinical trials have demonstrated that treatment with MDX-210 is well tolerated and that MDX-210 is both immunologically and clinically active. Optimization of the dose and schedule of MDX-210 and development of combination treatments with cytokines that modulate immune effector cells will greatly enhance the efficacy of this novel BsAb construct for treatment of tumours that overexpress **HER-2**/neu. We envision that MDX-210 will be effective for treating patients with tumors that overexpress **HER-2**/neu, especially in the minimal disease setting.

L22 ANSWER 45 OF 56 MEDLINE DUPLICATE 19

96129482 Document Number: 96129482. PubMed ID: 8581384. Clinical development of 2B1, a **bispecific** murine monoclonal antibody targeting c-erbB-2 and Fc gamma RIII. Weiner L M; Clark J I; Ring D B; Alpaugh R K. (Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA 19111, USA.) JOURNAL OF HEMATOTHERAPY, (1995 Oct) 4 (5) 453-6. Journal code: 9306048. ISSN: 1061-6128. Pub. country: United States. Language: English.

AB **Bispecific** monoclonal antibodies (BsmAb) can be used to specifically target tumor cells for cytotoxicity mediated by defined effector cells. One such BsmAb, 2B1, targets the extracellular domains of both the c-erbB-2 protein product of the **HER-2**/neu oncogene and Fc gamma RIII (CD16), the Fc gamma receptor expressed by human natural killer cells, neutrophils, and differentiated mononuclear phagocytes. 2B1 promotes the conjugation of cells expressing these target antigens. It efficiently promotes the specific lysis of tumor cells expressing c-erbB-2 by human NK cells and macrophages over a broad concentration range. 2B1 selectively targets c-erbB-2-positive human tumor xenografts growing in immunodeficient SCID mice. Treatment of such mice with 2B1 plus interleukin 2 (IL-2) inhibits the growth of early, established human tumor xenografts overexpressing c-erbB-2. A phase I clinical trial of 2B1 has been initiated to determine the toxicity profile and maximum tolerated dose (MTD) of this BsmAb and to examine the biodistribution of the antibody and the biologic effects of treatment. Preliminary results of this trial indicate that the dose-limiting toxicity for patients with extensive prior bone marrow-toxic therapy is thrombocytopenia for as yet undetermined reasons. Toxicities of fevers, rigors, and associated constitutional symptoms are explained, in part, by treatment-induced systemic expression of cytokines, such as tumor necrosis factor-alpha. Circulating, functional BsmAb is easily detectible in treatment patients' sera and exhibits complex elimination patterns. HAMA and anti-idiotypic treatment-induced antibodies are induced by 2B1 treatment. Some preliminary indications of clinical activity have been observed. BsmAb therapy targeting tumor antigens and Fc gamma RIII has potent immunologic effects. Future studies will include the development of more relevant animal models for BsmAb therapy targeting human Fc gamma RIII. The ongoing phase I trial will be completed to identify the MTD for patients without extensive prior bone marrow-toxic chemotherapy and radiation. A phase II clinical trial of 2B1 therapy in women with metastatic breast cancer is planned, as is a phase I trial incorporating treatment with both 2B1 and IL-2.

L22 ANSWER 46 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

1995:186765 Document No.: PREV199598201065. Targeting of **HER-2**/neu overexpressing breast and ovarian cancer with humanized **bispecific** F(ab')-2 alpha-p-185HER-2/alpha-CD3 and human LAK cells in vivo. Pegram, M. (1); Pietras, R. (1); Beryt, M. (1); Carter, P.; Shalaby, R.; Slamon, D. (1). (1) Div. Hematol. Oncol., UCLA Cent. Health

Sci., Los Angeles, CA USA. Proceedings of the American Association for Cancer Research Annual Meeting, (1995) Vol. 36, No. 0, pp. 433. Meeting Info.: Eighty-sixth Annual Meeting of the American Association for Cancer Research Toronto, Ontario, Canada March 18-22, 1995 ISSN: 0197-016X. Language: English.

L22 ANSWER 47 OF 56 MEDLINE DUPLICATE 20
96129476 Document Number: 96129476. PubMed ID: 8581378. G-CSF-stimulated PMN in immunotherapy of breast cancer with a **bispecific** antibody to Fc gamma RI and to **HER-2**/neu (MDX-210). Repp R; Valerius T; Wieland G; Becker W; Steininger H; Deo Y; Helm G; Gramatzki M; Van de Winkel J G; Lang N; +. (Department of Medicine III, University of Erlangen-Nurnberg, Germany.) JOURNAL OF HEMATOTHERAPY, (1995 Oct) 4 (5) 415-21. Ref: 26. Journal code: 9306048. ISSN: 1061-6128. Pub. country: United States. Language: English.

AB Myeloid cells can mediate tumor cell cytotoxicity via certain receptors for immunoglobulins. Among the different Fc receptors, the high-affinity IgG receptor (Fc gamma RI, CD64) is a promising trigger molecule because it is selectively expressed on effector cells, including monocytes/macrophages and granulocyte colony-stimulating factor (G-CSF)-primed neutrophils. In vitro, a **bispecific** antibody (BsAb) (MDX-210, constructed by chemically cross-linking F(ab') fragments of monoclonal antibody (mAb) 520C9 to **HER-2**/neu and F(ab') fragments of mAb 22 to Fc gamma RI) mediated effective lysis of **HER-2**/neu overexpressing breast cancer cell lines. **HER-2**/neu (c-erbB2) is overexpressed in approximately 30% of breast and ovarian carcinomas and is a target for immunotherapy in clinical trials. In vitro assays showed Fc gamma RI-positive neutrophils to constitute a major effector cell population during G-CSF therapy. Based on these preclinical data and a preceding study at Dartmouth (New Hampshire) with a single dose of MDX-210 alone, a combination of G-CSF and MDX-210 is tested in a phase I study in breast cancer patients. In this study, patients receiving G-CSF are treated with escalating single doses of MDX-210. This therapy was generally well tolerated by the treated patients, some of whom reacted with fever and short periods of chills, which were temporally related to elevated plasma levels of IL-6 and TNF-alpha. After MDX-210 application, a transient decrease in the total white blood count and absolute neutrophil count (ANC) was observed. During G-CSF application, isolated neutrophils were highly cytotoxic in the presence of MDX-210 in vitro. These data indicate a potential role for G-CSF and BsAb in immunotherapy.

L22 ANSWER 48 OF 56 CAPLUS COPYRIGHT 2002 ACS
1995:743582 Document No. 123:225223 Immunity to oncogenic proteins. Cheever, Martin A.; Disis, Mary L.; Bernhard, Helga; Gralow, Julie R.; Hand, Susan L.; Huseby, Eric S.; Qin, Hui Lian; Takahashi, Masazumi; Chen, Wei (Department of Medicine, University of Washington, Seattle, WA, 98195, USA). Immunological Reviews, 145, 33-59 (English) 1995. CODEN: IMRED2. ISSN: 0105-2896. Publisher: Munksgaard.

AB A review, with 79 refs. The authors discuss **HER-2**/neu and ras human antibody responses, **HER-2**/neu, ras and bcr-abl specific helper T cell responses, **HER-2**/neu, ras, and bcr-abl specific CTL responses, and the induction of **HER-2**/neu-specific immunity in human by treatment with **bispecific** antibody specific for **HER-2**/neu CD16.

L22 ANSWER 49 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1996:44942 Document No.: PREV199698617077. Phase I trial with a **bispecific** antibody to Fc-gamma-RI and to **HER-2**/NEU (MDXH210) in breast cancer using G-CSF stimulated PMNs as effector cells. Oetzel, C. (1); Repp, R. (1); Valerius, T. (1); Wieland, G.; Becker, W.; Steininger, H.; Deo, Y.; Van De Winkel, J. G. J.; Kalden, J.

R. (1); Gramatzki, M. (1). (1) Dep. Med. III, Erlangen Germany. Onkologie, (1995) Vol. 18, No. SUPPL. 2, pp. 29. Meeting Info.: Annual Congress of the German and Austrian Societies for Hematology and Oncology Hamburg, Germany October 8-11, 1995 ISSN: 0378-584X. Language: English.

L22 ANSWER 50 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1994:290816 Document No.: PREV199497303816. Generation and characterization of a **bispecific** monoclonal antibody anti CD3/anti **HER-2**. Nistico, P. (1); Digiesi, G. (1); Del Bello, D. (1); De Monte, L.; Venturo, I. (1); Nicotra, M. R.; Malavasi, F.; Natali, P. G. (1). (1) Regina Elena Cancer Inst., Rome Italy. Proceedings of the American Association for Cancer Research Annual Meeting, (1994) Vol. 35, No. 0, pp. 510. Meeting Info.: 85th Annual Meeting of the American Association for Cancer Research San Francisco, California, USA April 10-13, 1994 ISSN: 0197-016X. Language: English.

L22 ANSWER 51 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1995:47146 Document No.: PREV199598061446. G-CSF stimulated neutrophils as effector cells in immunotherapy with a **bispecific** antibody to Fc-gamma-RI and to **HER-2**/neu (MDX210): Preclinical studies. Repp, R.; Valerius, T.; Stockmeyer, B.; Elsaesser, D.; Gramatzki, M.; Kalden, J. R.. Dep. Med. III, Erlangen Germany. Immunobiology, (1994) Vol. 191, No. 2-3, pp. 250-251. Meeting Info.: XXVth Meeting of the Society of Immunology Konstanz, Germany September 21-24, 1994 ISSN: 0171-2985. Language: English.

L22 ANSWER 52 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1994:289090 Document No.: PREV199497302090. Phase la/lb trial of **bispecific** monoclonal antibody (BsAb) therapy (anti-**Her-2**/neu x anti-CD64) (MDX-210) for breast or ovarian cancers that over express **Her-2**/neu. Valone, F. H.; Kaufman, P. A.; Fanger, M. W.; Guyre, P. M.; Memoli, V.. Dartmouth-Hitchock Med. Cent., Lebanon, NH USA. Proceedings of the American Association for Cancer Research Annual Meeting, (1994) Vol. 35, No. 0, pp. 220. Meeting Info.: 85th Annual Meeting of the American Association for Cancer Research San Francisco, California, USA April 10-13, 1994 ISSN: 0197-016X. Language: English.

L22 ANSWER 53 OF 56 MEDLINE
95076843 Document Number: 95076843. PubMed ID: 7985544. Towards an immunotherapy for p185HER2 overexpressing tumors. Carter P; Rodrigues M L; Lewis G D; Figari I; Shalaby M R. (Department of Protein Engineering, Genentech Inc., South San Francisco, CA 94080.) ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY, (1994) 353 83-94. Journal code: 0121103. ISSN: 0065-2598. Pub. country: United States. Language: English.

L22 ANSWER 54 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 21
1994:204119 Document No.: PREV199497217119. **Bispecific** monoclonal antibody therapy (anti **HER-2**/neu x anti-CD 64) for human breast cancers that overexpress **HER-2**/neu. Valone, Frank H. (1); Kaufman, Peter A. (1); Fanger, Michael W.; Guyre, Paul M.; Springgate, Clark. (1) Dartmouth-Hitchcock Med. Centre, Dep. Med., Lebanon, NH 03756 USA. Journal of Cellular Biochemistry Supplement, (1993) Vol. 0, No. 17G, pp. 255-256. Meeting Info.: Workshop on Chemoprevention of Breast Cancer: Surrogate Endpoints and Agents in Short-Term Clinical Trials Lake Tahoe, California, USA October 6-10, 1993 ISSN: 0733-1959. Language: English.

L22 ANSWER 55 OF 56 MEDLINE
93383500 Document Number: 93383500. PubMed ID: 8396823. Cell surface receptors and **bispecific** monoclonal antibodies: the link between basic science and medical oncology. Malavasi F; De Monte L B; Funaro A;

Magrini E; Bonino L D; Momo M; Mariani M; Horenstein A; Natali P G. (Departimento di Genetica, Biologia e Chimica Medica, Universita di Torino, Italia.) YEAR IN IMMUNOLOGY, (1993) 7 74-80. Ref: 15. Journal code: 8403229. ISSN: 0256-2308. Pub. country: Switzerland. Language: English.

L22 ANSWER 56 OF 56 MEDLINE DUPLICATE 22
93090873 Document Number: 93090873. PubMed ID: 1457511. Biology and therapy with biologic agents in gynecologic cancer. Wiener J R; Berchuck A; Bast R C Jr. (Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, NC 27710.) CURRENT OPINION IN ONCOLOGY, (1992 Oct) 4 (5) 946-54. Ref: 52. Journal code: 9007265. ISSN: 1040-8746. Pub. country: United States. Language: English.

AB Growth of epithelial ovarian cancer is influenced by several factors including transforming growth factor-alpha and transforming growth factor-beta, macrophage colony stimulating factor, tumor necrosis factor-alpha, interleukin-1 and interleukin-6, c-erb B-2 (**HER-2**/neu), and mutant p53. Continued expression of the epidermal growth factor receptor, new expression of c-fms, and overexpression of **HER-2**/neu are associated with a poor prognosis. A number of cytokines have been used to treat patients with ovarian cancer, including interferon-alpha, interferon-gamma, tumor necrosis factor-alpha, and interleukin-2. Judging from preclinical models, interferon-gamma may be more active than interferon-alpha against human ovarian cancer. Although tumor necrosis factor-alpha can stimulate proliferation of some ovarian cancers, the cytotoxic activity of tumor necrosis factor-alpha has been amplified ex vivo by inhibitors of protein synthesis. Similar heterogeneity exists with regard to interleukin-1 where stimulation or inhibition of cell proliferation has been observed. Tumor-infiltrating lymphocytes from ascites fluid contain cells capable of major histocompatibility complex-restricted and major histocompatibility complex-nonrestricted cytotoxicity. Tumor-infiltrating lymphocytes and interleukin-2 have been combined with cytotoxic chemotherapy to treat advanced or recurrent disease. **Bispecific** monoclonal antibodies that react both with T cells and ovarian tumor cells have produced tumor inhibition in human tumor xenografts. Immunotoxins that contain OVB3 and pseudomonas exotoxin have been evaluated in a phase I clinical trial. Dose-limiting central neurotoxicity has been observed without tumor regression. A monoclonal antibody designated OVX1 has been developed against a high-molecular-weight mucinlike molecule associated with ovarian cancers.(ABSTRACT TRUNCATED AT 250 WORDS)

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FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 11:28:29 ON 22 DEC 2002

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L1            1 S BISPECIFIC POLYPEPTIDE
L2            4 S BISPECIFIC AND VEGF RECEPTOR
L3            4 DUP REMOVE L2 (0 DUPLICATES REMOVED)
L4          541 S BISPECIFIC AND CYTOKINE
L5           10 S L4 AND SINGLE CHAIN FV
L6            3 DUP REMOVE L5 (7 DUPLICATES REMOVED)
L7          279 DUP REMOVE L4 (262 DUPLICATES REMOVED)
L8           13 S L7 AND CYTOKINE RECEPTOR
L9           13 DUP REMOVE L8 (0 DUPLICATES REMOVED)
L10          16 S BISPECIFIC AND KDR
L11           6 DUP REMOVE L10 (10 DUPLICATES REMOVED)
L12           1 S BISPECIFIC AND FLK-1
L13           8 S BISPECIFIC AND FLT-1
L14           6 DUP REMOVE L13 (2 DUPLICATES REMOVED)
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L15             0 S BISPECIFIC AND FGF RECEPTOR
L16             0 S BISPECIFIC AND PDGF-R
L17            70 S BISPECIFIC AND EGF RECEPTOR
L18            27 DUP REMOVE L17 (43 DUPLICATES REMOVED)
L19             8 S L18 AND SINGLE CHAIN
L20             8 DUP REMOVE L19 (0 DUPLICATES REMOVED)
L21           125 S BISPECIFIC AND HER-2
L22            56 DUP REMOVE L21 (69 DUPLICATES REMOVED)

=> s l22 and single chain
L23             2 L22 AND SINGLE CHAIN

=> dup remove l23
PROCESSING COMPLETED FOR L23
L24             2 DUP REMOVE L23 (0 DUPLICATES REMOVED)

=> d l24 1-2 cbib abs

L24  ANSWER 1 OF 2     MEDLINE
2002083005  Document Number: 21668038.    PubMed ID: 11809717.   Adenovirus
     targeting to c-erbB-2 oncoprotein by single-chain
     antibody fused to trimeric form of adenovirus receptor ectodomain.
     Kashentseva Elena A; Seki Toshiro; Curiel David T; Dmitriev Igor P.
     (Division of Human Gene Therapy, Department of Medicine, University of
     Alabama at Birmingham, 35294-3300, USA. ) CANCER RESEARCH, (2002 Jan 15)
     62 (2) 609-16.  Journal code: 2984705R. ISSN: 0008-5472. Pub. country:
     United States. Language: English.
AB   The use of adenovirus (Ad) vectors for cancer gene therapy applications is
     currently limited by several factors, including broad Ad tropism
     associated with the widespread expression of coxsackievirus and adenovirus
     receptor (CAR) in normal human tissues, as well as limited levels of CAR
     in tumor cells. To target Ad to relevant cell types, we have proposed
     using soluble CAR (sCAR) ectodomain fused with a ligand to block
     CAR-dependent native tropism and to simultaneously achieve infection
     through a novel receptor overexpressed in target cells. To confer Ad
     targeting capability on cancer cells expressing the c-erbB-2/HER
     -2/neu oncogene, we engineered a bispecific adapter
     protein, sCARfC6.5, that consisted of sCAR, phage T4 fibritin polypeptide,
     and C6.5 single-chain fragment variable (scFv) against
     c-erbB-2 oncoprotein. Incorporation of fibritin polypeptide provided
     trimerization of sCAR fusion proteins that, compared with monomeric sCAR
     protein, resulted in augmented affinity to Ad fiber knob domain and in
     increased ability to block CAR-dependent Ad infection. We demonstrated
     that sCARfC6.5 protein binds to cellular c-erbB-2 oncoprotein and mediates
     efficient Ad targeting via a CAR-independent pathway. As illustrated in
     cancer cell lines that overexpress c-erbB-2, targeted Ad, complexed with
     sCARfC6.5 adapter protein, provided from 1.5- to 17-fold enhancement of
     gene transfer compared with Ad alone and up to 130-fold increase in
     comparison with untargeted Ad complexed with sCARf control protein. The
     use of recombinant trimeric sCAR-scFv adapter proteins may augment Ad
     vector potency for targeting cancer cell types.

L24  ANSWER 2 OF 2     MEDLINE
2001376626  Document Number: 21326013.    PubMed ID: 11433407.
     Bispecific single-chain antibodies as
     effective tools for eliminating epithelial cancer cells from human stem
     cell preparations by redirected cell cytotoxicity. Maletz K; Kufer P; Mack
     M; Raum T; Pantel K; Riethmuller G; Gruber R. (Institute for Immunology,
     Ludwig-Maximilians-Universitat Munchen, Munich, Germany. ) INTERNATIONAL
     JOURNAL OF CANCER, (2001 Aug 1) 93 (3) 409-16.  Journal code: 0042124.
     ISSN: 0020-7136. Pub. country: United States. Language: English.
AB   High-dose chemotherapy (HDC) with autologous bone marrow or peripheral
     stem cell transplantation is discussed as one option to treat the
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extensive stage of a variety of tumors. Effective methods to eliminate contaminating tumor cells from human bone marrow or stem cell grafts may improve the outcome of the patients. We investigated 3 recombinant **bispecific single-chain** antibodies (bscAbs) directed against 17-1A (EpCAM), c-erbB-2 (**HER-2**/neu) and LeY on the one and CD3 on the other binding site for their ability to induce lysis of epithelial tumor cells by retargeting autochthonous T lymphocytes present in bone marrow mononuclear cells (BMMC) and in peripheral stem cell mononuclear cells (PSMC). The bscAbs showed remarkable specific lysis of different epithelial tumor cell lines with BMMCs as well as with PSMCs as effector cells. Investigation of the alpha 17-1A-alpha CD3 bscAb revealed a significant correlation between the percentage of CD3(+) cells present in the BMMCs and the rate of lysis as well as the absence of detrimental effects on the viability of hematopoietic progenitor cells as determined by colony-forming unit assays (CFUs). Our results indicate that recombinant **bispecific single-chain** antibodies could be new tools for purging of human bone marrow and peripheral stem cell grafts from contaminating epithelial cancer cells for patients receiving autologous stem cell transplantation after HDC.


```
=> s bispecific and Tie-1
L25           0 BISPECIFIC AND TIE-1

=> s bispecific and Tek-2
L26           0 BISPECIFIC AND TEK-2

=> s bispecific and single chain Fv
L27         279 BISPECIFIC AND SINGLE CHAIN FV

=> dup remove l27
PROCESSING COMPLETED FOR L27
L28         128 DUP REMOVE L27 (151 DUPLICATES REMOVED)

=> s l28 and different specificity
L29           4 L28 AND DIFFERENT SPECIFICITY

=> dup remove l29
PROCESSING COMPLETED FOR L29
L30           4 DUP REMOVE L29 (0 DUPLICATES REMOVED)

=> d l30 1-4 cbib abs

L30  ANSWER 1 OF 4     MEDLINE
2000295268  Document Number: 20295268.    PubMed ID: 10835110.   An efficient
     route to the production of an IgG-like bispecific antibody. Zuo
     Z; Jimenez X; Witte L; Zhu Z. (Department of Molecular and Cell Biology,
     ImClone Systems Incorporated, 180 Varick Street, New York, NY 10014, USA.
     ) PROTEIN ENGINEERING, (2000 May) 13 (5) 361-7.  Journal code: 8801484.
     ISSN: 0269-2139. Pub. country: ENGLAND: United Kingdom. Language: English.
AB   Production of IgG-form bispecific antibody (BsAb-IgG) by
     co-expressing two antibodies in transfected cells is often inefficient
     owing to the unwanted pairing between the component heavy and light
     chains. We have developed an efficient method for the production of a
     novel IgG-like BsAb by using the natural dimerization mechanism between
     IgG heavy and light chains. Two single-chain
     Fv (scFv) of different specificity are fused
     to the constant domain of human kappa chain (C(L)) and the first constant
     domain of human heavy chain (C(H1)), to form two polypeptides,
     (scFv)(1)-C(L) and (scFv)(2)-C(H1)-C(H2)-C(H3), respectively.
     Co-expression of the two polypeptides in mammalian cells results in the
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formation of a covalently linked IgG-like hetero-tetramer, Bs(scFv)(4)-IgG, with dual specificity. Our approach yields a homogeneous **bispecific** IgG-like antibody product with each molecule containing four antigen binding sites, two for each of its target antigens. A Bs(scFv)(4)-IgG was prepared using two scFv antibodies each directed against a different epitope of a vascular endothelial growth factor receptor, the kinase insert domain-containing receptor (KDR). The Bs(scFv)(4)-IgG is capable of simultaneously binding to the two epitopes on the receptor. Further, the Bs(scFv)(4)-IgG also retains the antigen-binding efficacy and biological activity of its component antibodies.

L30 ANSWER 2 OF 4 MEDLINE
1998374022 Document Number: 98374022. PubMed ID: 9710248. A dimeric **bispecific** miniantibody combines two specificities with avidity. Muller K M; Arndt K M; Pluckthun A. (Biochemisches Institut, Universitat Zurich, Switzerland.) FEBS LETTERS, (1998 Jul 31) 432 (1-2) 45-9. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB **Bispecific** antibodies extend the capabilities of nature and might be applied in immunotherapy and biotechnology. By fusing the gene of a **single-chain Fv** (scFv) fragment to a helical dimerization domain, followed by a second scFv fragment of **different specificity**, we were able to express a functional protein in E. coli, which is **bispecific** and has two valencies for each specificity. The dimeric **bispecific** (DiBi) miniantibody preserves the natural avidity of antibodies in a very small-sized molecule of only 120 kDa. The generality of the principle was shown with a scFv fragment binding the EGF-receptor (named scFv 425) in three combinations with scFv fragments either directed against CD2 (ACID2.M1), phosphorylcholine (McPC603) or fluorescein (FITC-E2). Binding was analyzed by sandwich surface plasmon resonance biosensor (BIAcore) measurements.

L30 ANSWER 3 OF 4 SCISEARCH COPYRIGHT 2002 ISI (R)
97:115363 The Genuine Article (R) Number: WE998. Design and production of novel tetravalent **bispecific** antibodies. Coloma M J; Morrison S L (Reprint). UNIV CALIF LOS ANGELES, DEPT MICROBIOL & MOL GENET, 405 HILGARD AVE, LOS ANGELES, CA 90095 (Reprint); UNIV CALIF LOS ANGELES, DEPT MICROBIOL & MOL GENET, LOS ANGELES, CA 90095; UNIV CALIF LOS ANGELES, INST MOL BIOL, LOS ANGELES, CA 90095. NATURE BIOTECHNOLOGY (FEB 1997) Vol. 15, No. 2, pp. 159-163. Publisher: NATURE PUBLISHING CO. 345 PARK AVE SOUTH, NEW YORK, NY 10010-1707. ISSN: 1087-0156. Pub. country: USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We have produced novel **bispecific** antibodies by fusing the DNA encoding a single chain antibody (ScFv) after the C terminus (C(H)3-ScFv) or after the hinge (Hinge-ScFv) with an antibody of a **different specificity**, The fusion protein is expressed by gene transfection in the context of a murine variable region, Transfectomas secrete a homogeneous population of the recombinant antibody with two **different specificities**, one at the N terminus (anti-dextran) and one at the C terminus (anti-dansyl). The C(H)3-ScFv antibody, which maintains the constant region of human IgG3, has some of the associated effector functions such as long half-life and Fc receptor binding, The Hinge-ScFv antibody which lacks the C(H)2 and C(H)3 domains has no known effector functions.

L30 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2002 ACS
1996:198737 Document No. 124:258003 Leucine zipper dimerized bivalent and **bispecific** scFv antibodies from a semi-synthetic antibody phage display library. de Kruif, John; Logtenberg, Ton (Dep. Immunology, Utrecht Univ., Utrecht, 3508 GA, Neth.). Journal of Biological Chemistry,

271(13), 7630-4 (English) 1996. CODEN: JBCHA3. ISSN: 0021-9258.
Publisher: American Society for Biochemistry and Molecular Biology.
AB This report describes the construction of leucine zipper-based dimerization cassettes for the conversion of recombinant monomeric scFv antibody fragments to bivalent and **bispecific** dimers. A truncated murine IgG3 hinge region and a Fos or Jun leucine zipper were cloned into four scFv fragments previously isolated from a synthetic antibody phage display library. Cysteine residues flanking the zipper region were introduced to covalently link dimerized scFv fragments. The secreted fusion proteins were shown to spontaneously and efficiently form stable Fos.cntdot.Fos or Jun.cntdot.Jun homodimers in the Escherichia coli periplasm at levels comparable to their monovalent counterparts. The bivalent (scFv)2 fragments performed well in ELISA, flow-cytometric, and immunohistochem. anal. Fos and Jun homodimer (scFv)2 antibodies with **different specificities** could be reduced, reshuffled, and reoxidized to form prepns. of functional **bispecific** (scFv)2 Fos.cntdot.Jun heterodimers. These Fos and Jun fusion protein cassettes provide a universal basis for the construction of dimeric scFv antibodies with enhanced avidity or dual specificity.

=> d his

(FILE 'HOME' ENTERED AT 11:28:17 ON 22 DEC 2002)

FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 11:28:29 ON 22 DEC 2002

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L1            1 S BISPECIFIC POLYPEPTIDE
L2            4 S BISPECIFIC AND VEGF RECEPTOR
L3            4 DUP REMOVE L2 (0 DUPLICATES REMOVED)
L4          541 S BISPECIFIC AND CYTOKINE
L5           10 S L4 AND SINGLE CHAIN FV
L6            3 DUP REMOVE L5 (7 DUPLICATES REMOVED)
L7          279 DUP REMOVE L4 (262 DUPLICATES REMOVED)
L8           13 S L7 AND CYTOKINE RECEPTOR
L9           13 DUP REMOVE L8 (0 DUPLICATES REMOVED)
L10          16 S BISPECIFIC AND KDR
L11           6 DUP REMOVE L10 (10 DUPLICATES REMOVED)
L12           1 S BISPECIFIC AND FLK-1
L13           8 S BISPECIFIC AND FLT-1
L14           6 DUP REMOVE L13 (2 DUPLICATES REMOVED)
L15           0 S BISPECIFIC AND FGF RECEPTOR
L16           0 S BISPECIFIC AND PDGF-R
L17          70 S BISPECIFIC AND EGF RECEPTOR
L18          27 DUP REMOVE L17 (43 DUPLICATES REMOVED)
L19           8 S L18 AND SINGLE CHAIN
L20           8 DUP REMOVE L19 (0 DUPLICATES REMOVED)
L21         125 S BISPECIFIC AND HER-2
L22          56 DUP REMOVE L21 (69 DUPLICATES REMOVED)
L23           2 S L22 AND SINGLE CHAIN
L24           2 DUP REMOVE L23 (0 DUPLICATES REMOVED)
L25           0 S BISPECIFIC AND TIE-1
L26           0 S BISPECIFIC AND TEK-2
L27         279 S BISPECIFIC AND SINGLE CHAIN FV
L28         128 DUP REMOVE L27 (151 DUPLICATES REMOVED)
L29           4 S L28 AND DIFFERENT SPECIFICITY
L30           4 DUP REMOVE L29 (0 DUPLICATES REMOVED)
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=> d l28 1-128 cbib abs

L28 ANSWER 1 OF 128 CAPLUS COPYRIGHT 2002 ACS
2002:794190 Document No. 137:309493 Antibody specific to CD14 preparation and uses thereof. Devitt, Andrew; Gregory, Christopher Darrell; Sanghera,

Jaspal; Pierce, Sarah Elizabeth (UK). U.S. Pat. Appl. Publ. US 2002150882 A1 20021017, 25 pp. (English). CODEN: USXXCO. APPLICATION: US 2001-835756 20010416.

AB The authors disclose a method for prepn. of antibodies Ce3, R7, or R29, where Ce3 is capable of binding the cell membrane-assocd. CD14 and the other 2 sol. CD14. These reagents can be used to identify and/or manipulate the actions of CD14 on the cells expressing it. Ce3, R7, or R29 are expressed by bacterial strains with the accession nos. NCIMB 41088, NCIMB 41086, and NCIMB 41087, resp. The authors present a scheme illustrating a semi-synthetic phage antibody display library Lib (Kruif, et al., 1995), which comprises a phagemid with addnl. elements, the selection of antibodies that bind CD14, and use of ELISA technol. to select phage antibodies.

L28 ANSWER 2 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

2002:419008 Document No.: PREV200200419008. Production and evaluation of **bispecific single-chain Fv** molecules that target HER2/neu and HER3. Horak, Eva M. (1); Shahied, Lillian S.; Shaller, Calvin C.; Tesfaye, Abohawariat; Simmons, Heidi H.; Alpaugh, R. Katherine; Greer, Nathaniel B.; Heitner, Tara; Garrison, Jennifer L.; Marks, James D.; Weiner, Louis M.; Adams, Gregory P.. (1) Fox Chase Cancer Center, Philadelphia, PA USA. Proceedings of the American Association for Cancer Research Annual Meeting, (March, 2002) Vol. 43, pp. 971. print. Meeting Info.: 93rd Annual Meeting of the American Association for Cancer Research San Francisco, California, USA April 06-10, 2002 ISSN: 0197-016X. Language: English.

L28 ANSWER 3 OF 128 MEDLINE

2002711343 Document Number: 22361431. PubMed ID: 12473192. Efficient construction of a diabody using a refolding system: anti-carcinoembryonic antigen recombinant antibody fragment. Asano Ryutaro; Kudo Toshio; Nishimura Yukihiro; Makabe Koki; Hayashi Hiroki; Suzuki Masanori; Tsumoto Kouhei; Kumagai Izumi. (Department of Biomolecular Engineering, Graduate School of Engineering, Tohoku University, Aoba-ku, Sendai 980-8579, Japan.. kmiz@mail.cc.tohoku.ac.jp) . JOURNAL OF BIOCHEMISTRY, (2002 Dec) 132 (6) 903-9. Journal code: 0376600. ISSN: 0021-924X. Pub. country: Japan. Language: English.

AB Recombinant fragments of the variable region of antibodies are useful in many experimental and clinical applications. However, it can be difficult to obtain these materials in soluble form after their expression in bacteria. Here, we report an efficient procedure for preparing several variable-domain fragments (Fv), **single-chain Fv** (scFv), and a diabody (the smallest functional **bispecific** antibody) of anti-carcinoembryonic antigen (CEA) antibody by overexpression in Escherichia coli in inclusion bodies, using a refolding system to obtain renatured proteins. Two types of refolded Fv were prepared: (i) Heavy and light chains of the immunoglobulin variable regions (VH and VL, respectively) were coexpressed with a dicistronic expression vector (designated Fv(co)); (ii) VH and VL were expressed separately, mixed stoichiometrically, and refolded (designated Fv(mix)). All samples refolded with high efficiency; Fv(co), Fv(mix), scFv, and the **bispecific** diabody bound to several CEA-positive cell lines, exactly as did soluble Fv fragments secreted by E. coli (Fv(sol)) and the parent IgG. The refolded fragments inhibited binding of the parent IgG to CEA-positive cell lines, indicating that their epitope is identical to that of IgG. The **bispecific** diabody, which combined variable-region fragments of anti-CEA antibody with variable-region fragments of anti-CD3 antibody, was also prepared using the refolding system. This refolded diabody could bind to lymphokine-activated killer cells. In addition, its cytotoxicity toward human bile duct carcinoma TFK-1 and other several other CEA-positive cell lines was concentration-dependent. Taken together, our results suggest that a refolding procedure can be used to prepare various functional antibody

fragments (Fv, scFv, and diabody).

L28 ANSWER 4 OF 128 MEDLINE DUPLICATE 1
2002449845 Document Number: 22198201. PubMed ID: 12209608. Extremely potent, rapid and costimulation-independent cytotoxic T-cell response against lymphoma cells catalyzed by a single-chain **bispecific** antibody. Dreier Torsten; Lorenczewski Grit; Brandl Christian; Hoffmann Patrick; Syring Uwe; Hanakam Frank; Kufer Peter; Riethmuller Gert; Bargou Ralf; Baeuerle Patrick A. (Micromet AG, Am Klopferspitz 19, 82152 Martinsried, Germany.) INTERNATIONAL JOURNAL OF CANCER, (2002 Aug 20) 100 (6) 690-7. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB A recent study reported on an anti-CD19/anti-CD3 single-chain **bispecific** antibody (bscCD19xCD3) exhibiting high activity against human B lymphoma cell lines (Loffler et al., Blood 2000;95:2098-103). In the present study, we have explored in detail the in vitro efficacy, T-cell donor variability, binding characteristics, specificity, kinetics and interleukin-2 (IL-2) dependence of bscCD19xCD3. We found that a majority of human donor T cells tested (n = 86) gave half-maximal B-lymphoma cell lysis (ED(50)) within a range of 10-50 pg/ml bscCD19xCD3, corresponding to sub-picomolar concentrations of the **bispecific** antibody. Under identical experimental conditions, the anti-CD20 monoclonal antibody rituximab had an at least 100,000-fold lower in vitro efficacy. The extreme potency of bscCD19xCD3 was in sharp contrast to the relatively low affinity of the anti-CD3 and anti-CD19 **single-chain Fv** portions in K(D) ranges of 10(-7) and 10(-9) M, respectively. Cell lysis by bscCD19xCD3 was predominantly mediated by the population of CD8/CD45RO-positive T cells. Both immortalized CD4- and CD8-positive human T-cell clones were highly active effector cells as well. Cell lysis by bscCD19xCD3 was rapid and specific. The respective parental monoclonal antibodies inhibited cell lysis and CD19-negative cells were not harmed by T cells in the presence of high amounts of bscCD19xCD3. The potent T-cell stimulus IL-2 could not markedly augment the activity of bscCD19xCD3-stimulated T cells. In conclusion, bscCD19xCD3 could redirect unstimulated cytotoxic T cells against CD19-positive cells in an unexpectedly potent, rapid and specific fashion.

L28 ANSWER 5 OF 128 MEDLINE DUPLICATE 2
2002308226 Document Number: 22045580. PubMed ID: 12050554. Epidermal growth factor receptor targeting of replication competent adenovirus enhances cytotoxicity in bladder cancer. van der Poel H G; Molenaar B; van Beusechem V W; Haisma H J; Rodriguez R; Curiel D T; Gerritsen W R. (Department of Urology, The Netherlands Cancer Institute, Amsterdam, The Netherlands.) JOURNAL OF UROLOGY, (2002 Jul) 168 (1) 266-72. Journal code: 0376374. ISSN: 0022-5347. Pub. country: United States. Language: English.

AB PURPOSE: We evaluated the delivery and oncolytic potential of targeted replication competent adenoviruses in bladder cancer lines. MATERIALS AND METHODS: Seven established human bladder cancer tumor lines (5637, SW800, TCCsup, J82, Scaber, T24 and 253J) were studied for the expression of integrins alpha(v)beta3, alpha(v)beta5, Coxsackievirus and adenovirus receptor, epidermal growth factor receptor (EGF-R) and epithelial cell adhesion molecule antigens using flow cytometry analysis. **Bispecific single chain Fv** fragments were used to target replication deficient luciferase reporter adenovirus to EGF-R (425-s11) or to epithelial cell adhesion molecule (C28-s11) antigens. Moreover, a fiber modified adenovirus targeting alpha(v)-integrins was studied. Replication competent serotype-5 adenoviruses attenuated to replicate specifically in retinoblastoma pRb (Ad5-d24) or p53 deficient (Ad5-d55K) cells were tested in vitro for oncolytic properties. RESULTS: Low to absent Coxsackievirus and adenovirus receptor expression was found in 5 of the 7 tumor lines (SW800, J82, T24,

5637 and Scaber). EGF-R expression was found in all cell lines, whereas elevated epithelial cell adhesion molecule expression was seen in 3 (5637, Scaber and TCCsup), alpha(v)beta3-integrin was found in 1 (Scaber) and alpha(v)beta5-integrin was found in 3 (TCCsup, 253J and T24). EGF-R targeting using 425-s11 improved transgene expression in all cell lines from 2.1 to 12.5 times over nontargeted viruses. Epithelial cell adhesion molecule and integrin targeting was inferior to EGF-R targeting with a maximal increase in transgene expression of 2 times for epithelial cell adhesion molecule in 5637cells and 1.6 times for integrin targeting in T24 cells. Comparison of the wild-type replication competent virus with conditionally replicating adenoviruses (Ad5-d55K and Ad5-d24) showed superior oncolytic activity for the latter 2 in all lines. Furthermore, improved cytotoxicity (29% to 33%) was obtained in 4 of the 7 lines after pre-incubation of Ad5-d24 with 425-s11. CONCLUSIONS: EGF-R directed **bispecific** single chain antibodies enhance adenovirus mediated transgene expression and oncolysis in bladder cancer lines.

L28 ANSWER 6 OF 128 MEDLINE DUPLICATE 3

2002411062 Document Number: 22155357. PubMed ID: 12165442. Fab-scFv fusion protein: an efficient approach to production of **bispecific** antibody fragments. Lu Dan; Jimenez Xenïa; Zhang Haifan; Bohlen Peter; Witte Larry; Zhu Zhenping. (Department of Antibody Technology, ImClone Systems Incorporated, 180 Varick Street, New York, NY 10014, USA.) JOURNAL OF IMMUNOLOGICAL METHODS, (2002 Sep 15) 267 (2) 213-26. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB The clinical development of **bispecific** antibodies (BsAb) as therapeutics has been hampered by the difficulty in preparing the materials in sufficient quantity and quality by traditional methods. Here, we describe an efficient approach for the production of a novel **bispecific** antibody fragment by genetically fusing a **single-chain Fv** (scFv) to the C-terminus of either the light chain or the heavy chain of a Fab fragment of different antigen-binding specificity. The **bispecific** Fab-scFv fragments were expressed in a single Escherichia coli host and purified to homogeneity by a one-step affinity chromatography. Two different versions of the **bispecific** Fab-scFv fragments were constructed using two antibodies directed against the two tyrosine kinase receptors of vascular endothelial growth factor. These **bispecific** antibody fragments not only retained the antigen-binding capacity of each of the parent antibodies, but also are capable of binding to both targets simultaneously as demonstrated by a cross-linking ELISA. Further, the **bispecific** antibodies were comparable to their parent antibodies in their potency in blocking ligand binding to the receptors and in inhibiting ligand-induced biological activities. This design for BsAb fragments should be applicable to any pair of antigen specificities.

L28 ANSWER 7 OF 128 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

2002070403 EMBASE A mutated superantigen SEA D227A fusion diabody specific to MUC1 and CD3 in targeted cancer immunotherapy for bile duct carcinoma. Takemura S.-I.; Kudo T.; Asano R.; Suzuki M.; Tsumoto K.; Sakurai N.; Katayose Y.; Kodama H.; Yoshida H.; Ebara S.; Saeki H.; Imai K.; Matsuno S.; Kumagai I.. T. Kudo, Cell Resource Ctr. for Biomed. Res., Inst. Development, Aging/Cancer, Tohoku University, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan. j23700@gen.cc.tohoku.ac.jp. Cancer Immunology, Immunotherapy 51/1 (33-44) 2002.
Refs: 53.
ISSN: 0340-7004. CODEN: CIIMDN. Pub. Country: Germany. Language: English. Summary Language: English.

AB In cancer immunotherapy research, many **bispecific** antibodies (BsAbs) have been developed for directing T cells toward tumor cells. Recent advances in genetic engineering have made it possible to prepare immunoglobulin fragments consisting of variable domains using bacterial

expression systems. Therefore, recombinant BsAbs, termed diabodies, have attracted particular attention. We have previously produced an anti-MUC1 x anti-CD3 diabody (Mx3 diabody) in an Escherichia coli (E. coli) expression system. In order to reinforce the antitumor effects of the Mx3 diabody, mutated superantigen staphylococcal enterotoxin A (SEA) D227A was genetically fused to the Mx3 diabody. The SEA D227A fusion Mx3 diabody (SEA D227A-Mx3 diabody) thus constructed showed remarkable MUC1-specific antitumor effects when used with effector cells (lymphokine-activated killer cells with T-cell phenotype [T-LAK] and peripheral blood mononuclear cells [PBMCs]). In the bile duct carcinoma (BDC)-xenografted severe combined immunodeficient (SCID) mouse model, it also demonstrated strong antitumor activity when administered i.v. together with T-LAK cells and interleukin-2 (IL-2). In this experiment, the complete disappearance of tumors was observed in 3 out of 6 mice, and the other 3 showed marked retardation of tumor growth. Therefore, the SEA D227A-Mx3 diabody is considered to be a promising reagent in specific targeted immunotherapy for BDC and other MUC1- positive carcinomas. This is the first report on a diabody that is effective in treating human solid cancers in the xenografted SCID mouse experimental model.

L28 ANSWER 8 OF 128 MEDLINE DUPLICATE 4
2001448611 Document Number: 21240674. PubMed ID: 11342630. Increasing the affinity for tumor antigen enhances **bispecific** antibody cytotoxicity. McCall A M; Shahied L; Amoroso A R; Horak E M; Simmons H H; Nielson U; Adams G P; Schier R; Marks J D; Weiner L M. (Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA 19111, USA.) JOURNAL OF IMMUNOLOGY, (2001 May 15) 166 (10) 6112-7. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB We tested the hypothesis that **bispecific** Abs (Bsab) with increased binding affinity for tumor Ags augment retargeted antitumor cytotoxicity. We report that an increase in the affinity of Bsab for the HER2/neu Ag correlates with an increase in the ability of the Bsab to promote retargeted cytotoxicity against HER2/neu-positive cell lines. A series of anti-HER2/neu extracellular domain-directed **single-chain Fv** fragments (scFv), ranging in affinity for HER2/neu from 10(-7) to 10(-11) M, were fused to the phage display-derived NM3E2 human scFV: NM3E2 associates with the extracellular domain of human FcgammaRIII (CD16). The resulting series of Bsab promoted cytotoxicity of SKOV3 human ovarian carcinoma cells overexpressing HER2/neu by human PBMC preparations containing CD16-positive NK cells. The affinity for HER2/neu clearly influenced the ability of the Bsab to promote cytotoxicity of (51)Cr-labeled SKOV3 cells. Lysis was 6.5% with an anti-HER2/neu K(D) = 1.7 x 10(-7) M, 14.5% with K(D) = 5.7 x 10(-9) M, and 21.3% with K(D) = 1.7 x 10(-10) M at 50:1 E:T ratios. These scFv-based Bsab did not cross-link receptors and induce leukocyte calcium mobilization in the absence of tumor cell engagement. Thus, these novel Bsab structures should not induce the dose-limiting cytokine release syndromes that have been observed in clinical trials with intact IgG BSAB: Additional manipulations in Bsab structure that improve selective tumor retention or facilitate the ability of Bsab to selectively cross-link tumor and effector cells at tumor sites should further improve the utility of this therapeutic strategy.

L28 ANSWER 9 OF 128 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 5
2001246443 EMBASE Generation and characterization of a novel tetravalent **bispecific** antibody that binds to hepatitis B virus surface antigens. Sung Sup Park; Chun Jeih Ryu; Young Jun Kang; Kashmiri S.V.S.; Hyo Jeong Hong. H.J. Hong, Antibody Engineering Research Lab., KOR Res. Inst. Biosci./Biotechnol., PO Box 115, Yuseong, Taejon 305-600, Korea, Republic of. hjhong@mail.kribb.re.kr. Molecular Immunology 37/18 (1123-1130) 2001.
Refs: 29.
ISSN: 0161-5890. CODEN: IMCHAZ.

Publisher Ident.: S 0161-5890(01)00027-X. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB Hepatitis B virus (HBV) infection is a worldwide public health problem affecting about 350 million people. HBV envelope contains three surface antigens, called pre-S1, pre-S2 and S. For the prophylaxis of HBV infection, only an anti-S monoclonal antibody was tested for the protective efficacy against HBV infection, but it was shown to be incomplete. In addition, some immune escape mutants carrying mutations on the S antigen were reported. Therefore, a multivalent **bispecific** antibody rather than a single monoclonal antibody would be more beneficial for the prophylaxis of HBV infection. We have generated a novel tetravalent **bispecific** antibody with two binding sites for each of the S and pre-S2 antigens. Each of the antigen-binding sites was composed of a **single-chain Fv** (ScFv). The tetravalent antibody was generated by constructing a single gene encoding a single-chain protein. This protein consisted of an anti-S ScFv whose carboxyl end was tethered, through a 45 amino acid linker, to the amino terminus of anti-preS2 ScFv that in turn was joined to the hinge region of human .gamma.1 constant region. The single-chain protein was expressed in Chinese hamster ovary cells and secreted in culture supernatant as a homodimeric molecule. The tetravalent **bispecific** antibody showed both anti-S and anti-pre-S2 binding activities. In addition, the binding affinity of the **bispecific** antiboy for HBV particles was greater than that of either parental antibody. The tetravalent **bispecific** antibody is a potentially useful reagent for the prevention and treatment of HBV infection. .COPYRGT. 2001 Elsevier Science Ltd.

L28 ANSWER 10 OF 128 MEDLINE DUPLICATE 6

2001345693 Document Number: 21301860. PubMed ID: 11407902. Targeting of adenovirus to endothelial cells by a **bispecific** single-chain diabody directed against the adenovirus fiber knob domain and human endoglin (CD105). Nettelbeck D M; Miller D W; Jerome V; Zuzarte M; Watkins S J; Hawkins R E; Muller R; Kontermann R E. (Institut fur Molekularbiologie und Tumorforschung, Philipps-Universitat Marburg, Emil-Mannkopff-Strabetae 2, Marburg, D-35033, Germany.) MOLECULAR THERAPY, (2001 Jun) 3 (6) 882-91. Journal code: 100890581. ISSN: 1525-0016. Pub. country: United States. Language: English.

AB The use of adenoviruses for antivascular cancer gene therapy is limited by their low transduction efficiency for endothelial cells. We have developed a recombinant **bispecific** antibody as a molecular bridge, linking the adenovirus capsid to the endothelial cell surface protein endoglin, for vascular targeting of adenoviruses. Endoglin (CD105), a component of the transforming growth factor beta receptor complex, represents a promising target for antivascular cancer therapy. Endoglin is expressed predominantly on endothelial cells and is upregulated in angiogenic areas of tumors. We isolated **single-chain Fv** fragments directed against human endoglin from a human semisynthetic antibody library. One of the isolated scFv fragments (scFv C4) bound specifically to various proliferating primary endothelial cells or cell lines including HUVEC, HDMEC, HMVEC, and HMEC. ScFv C4 was therefore used to construct a **bispecific** single-chain diabody directed against endoglin and the adenovirus fiber knob domain (scDb EDG-Ad). This **bispecific** molecule mediated enhanced and selective adenovirus transduction of HUVECs, which was independent from binding to the coxsackievirus and adenovirus receptor (CAR) and alpha(v)-integrins. Thus, adenovirus infection was redirected to a new cellular receptor (CD105) and cell entry pathway. These results demonstrate the utility of **bispecific** single-chain diabodies, which can be produced in large quantities in bacteria, for the retargeting of adenoviruses in cancer gene therapy.

L28 ANSWER 11 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)

2001:941966 The Genuine Article (R) Number: 493YA. Recombinant antibodies for

cancer diagnosis and therapy. Souriau C; Hudson P J (Reprint). CSIRO Hlth Sci & Nutr, CRC Diagnost, 343 Royal Parade, Parkville, Vic 3052, Australia (Reprint); CSIRO Hlth Sci & Nutr, CRC Diagnost, Parkville, Vic 3052, Australia. EXPERT OPINION ON BIOLOGICAL THERAPY (SEP 2001) Vol. 1, No. 5, pp. 845-855. Publisher: ASHLEY PUBLICATIONS LTD. UNITEC HOUSE, 3RD FL, 2 ALBERT PLACE FINCHLEY CENTRAL, LONDON N3 1QB, ENGLAND. ISSN: 1471-2598. Pub. country: Australia. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Recombinant antibodies now represent over 30% of biopharmaceuticals in clinical trials, highlighted by the recent approvals for cancer immunotherapy from the FDA which has awoken the biotechnology industry. Sales of these antibodies are increasing very rapidly to a predicted US$ 3 billion per annum worldwide by 2002. Since the development of new therapeutic reagent into commercial product takes 10 years, the recent FDA-approved antibodies are based on early antibody designs which are now considered primitive. Emerging technologies have created a vast range of novel, recombinant, antibody-based reagents which specifically target clinical biomarkers, of disease. In the past year, radiolabelling of antibodies has increased their potential for cancer imaging and targeting. Recombinant antibodies have also been reduced in size and rebuilt into multivalent molecules for higher affinity. in addition, antibodies have been fused with many molecules including toxins, enzymes and viruses for prodrug therapy, cancer treatment and gene delivery. Recombinant antibody technology has enabled clever manipulations in the construction of complex antibody library repertoires for the selection of high-affinity reagents against refractory targets. Although phage display remains the most extensively used method, this year high affinity reagents have been isolated using alternative display and selection systems such as ribosome display and yeast display confirming the emergence of new display methods. Furthermore, innovative affinity maturation strategies have been developed to obtain high affinity reagents. This review focuses on developments in the last 12 months and describes the latest developments in the design, production and clinical use of recombinant antibodies for cancer diagnosis and therapy.

L28 ANSWER 12 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2002:1515 Document No.: PREV200200001515. Engineering **bispecific single-chain Fv** molecules to alter signaling of the EGF receptor family. Horak, Eva M. (1); Heitner, Tara; Garrison, Jennifer L.; Simmons, Heidi H.; Alpaugh, R. Katherine; Amoroso, Anne R.; Marks, James D.; Weiner, Louis M.; Adams, Gregory P.. (1) Fox Chase Cancer Center, Philadelphia, .PA USA. Proceedings of the American Association for Cancer Research Annual Meeting, (March, 2001) Vol. 42, pp. 774. print. Meeting Info.: 92nd Annual Meeting of the American Association for Cancer Research New Orleans, LA, USA March 24-28, 2001 ISSN: 0197-016X. Language: English.

L28 ANSWER 13 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
2001:825009 The Genuine Article (R) Number: 480NH. Specific immunotherapy of cancer in elderly patients. Matzku S; Zoller M (Reprint). German Canc Res Ctr, Dept Tumor Progress & Immune Def, Neuenheimer Feld 280, D-69120 Heidelberg, Germany (Reprint); German Canc Res Ctr, Dept Tumor Progress & Immune Def, D-69120 Heidelberg, Germany; Merck KGaA, Dept Oncol Biomed Res, Darmstadt, Germany; Univ Karlsruhe, Dept Appl Genet, Karlsruhe, Germany. DRUGS & AGING (1 SEP 2001) Vol. 18, No. 9, pp. 639-664. Publisher: ADIS INTERNATIONAL LTD. 41 CENTORIAN DR, PRIVATE BAG 65901, MAIRANGI BAY, AUCKLAND 10, NEW ZEALAND. ISSN: 1170-229X. Pub. country: Germany. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The concept of immunotherapy of cancer is more than a century old, but only recently have molecularly defined therapeutic approaches been developed. In this review, we focus on the most promising approach, active therapeutic vaccination.

The identification of tumour antigens can now be accelerated by methods allowing the amplification of gene products selectively or preferentially transcribed in the tumour. However, determining the potential immunogenicity of such gene products remains a demanding task, since major histocompatibility complex (MHC) restriction of T cells implies that for any newly defined antigen, immunogenicity will have to be defined for any individual MHC haplotype. Tumour-derived peptides elated from MHC molecules of tumour tissue are also a promising source of antigen.

Tumour antigens are mostly of weak immunogenicity, because the vast majority are tumour-associated differentiation antigens already 'seen' by the patient's immune system. Effective therapeutic vaccination will thus require adjuvant support, possibly by new approaches to immunomodulation such as **bispecific** antibodies or antibody-cytokine fusion proteins. Tumour-specific antigens, which could be a more potent target for immunotherapy, mostly arise by point mutations and have the disadvantage of being not only tumour-specific, but also individual-specific. Therapeutic vaccination will probably focus on defined antigens offered as protein, peptide or nucleic acid. Irrespective of the form in which the antigen is applied, emphasis will be given to the activation of dendritic cells as professional antigen presenters. Dendritic cells may be loaded in vitro with antigen, or, alternatively, initiation of an immune response may be approached in vivo by vaccination with RNA or DNA, given as such or packed into attenuated bacteria.

The importance of activation of T helper cells has only recently been taken into account in cancer vaccination. Activation of cytotoxic T cells is facilitated by the provision of T helper cell-derived cytokines. T helper cell-dependent recruitment of elements of non-adaptive defence, such as leucocytes, natural killer cells and monocytes, is of particular importance when the tumour has lost MHC class I expression.

Barriers to successful therapeutic vaccination include: (i) the escape mechanisms developed by tumour cells in response to immune attack; (ii) tolerance or anergy of the evoked immune response; (iii) the theoretical possibility of provoking an autoimmune reaction by vaccination against tumour-associated antigens; and (iv) the advanced age of many patients, implying reduced responsiveness of the senescent immune system.

L28 ANSWER 14 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)

2002:32407 The Genuine Article (R) Number: 507HT. Anti-HLA-DR/anti-DOTA diabody construction in a modular gene design platform: **Bispecific** antibodies for pretargeted radioimmunotherapy. DeNardo D G; Xiong C Y; Shi X B; DeNardo G L; DeNardo S J (Reprint). Univ Calif Davis, Ctr Med, Sect Radiodiagnosis & Therapy, Dept Internal Med, 1508 Alhambra Blvd, Suite 3100, Sacramento, CA 95816 USA (Reprint); Univ Calif Davis, Ctr Med, Sect Radiodiagnosis & Therapy, Dept Internal Med, Sacramento, CA 95816 USA . CANCER BIOTHERAPY AND RADIOPHARMACEUTICALS (DEC 2001) Vol. 16, No. 6, pp. 525-535. Publisher: MARY ANN LIEBERT INC PUBL. 2 MADISON AVENUE, LARCHMONT, NY 10538 USA. ISSN: 1084-9785. Pub. country: USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Recombinant immunoglobulin libraries of single chain molecules (sc) from the variable domains of antibody light and heavy chains (Fv), have great promise for new approaches to radio immunotherapy (RIT). However, creating and evaluating scFv from diverse sources is time consuming and differences in molecular-format can influence in vitro and in vivo characteristics. Furthermore, scFv do not have optimal characteristics for targeting therapy to tumor because of their small size and univalent binding. Diabody molecules at least twice the size of scFv are better for RIT because bivalent and **bispecific** molecules can be constructed.

A polymerase chain reaction (PCR) based primer system was created to easily convert scFv genes into a diabody gene format, once they have been placed into pCANTAB 5E, a readily available vector. The primer system for this diabody gene platform was developed and tested by constructing an

anti-lymphoma/anti-chelate, **bispecific** diabody (anti-HLA-DR/anti-DOTA). Two mouse scFv libraries were screened for reactive clones using recombinant phage display techniques. Selected mouse anti-HLA-DR and anti-DOTA scFv genes were combined, ligated into the pCANTAB 5E vector that co-expressed these self-assembling scFv in E. coli as two mismatched nonlinked pairs (V(H)A-link-VLB; VHB-link-V(L)A). The diabody protein that was purified from periplasm had the expected molecular characteristics when analyzed by sequencing, chromatography, electrophoresis and Western blot. This modular gene design platform provides methodology for easy and rapid creation of diabody molecules from diverse scFv libraries. Diabodies from various scFv can easily be produced, thereby facilitating comparative preclinical studies en route to development of new tumor targeting molecules.

L28 ANSWER 15 OF 128 CAPLUS COPYRIGHT 2002 ACS
2001:554171 Document No. 136:277768 Construction and expression of anti-HBsAg and anti-RBC **bispecific** minibody. Chen, Yuping; Qiao, Yuanyuan; Hua, Bing; Liu, Xiaolin; Xie, Bangtie; Ma, Dalong; Wang, Yan (Navy General Hospital, Beijing, 100037, Peop. Rep. China). Zhongguo Mianyixue Zazhi, 17(6), 298-301 (Chinese) 2001. CODEN: ZMZAEE. ISSN: 1000-484X. Publisher: Zhongguo Mianyixue Zazhi Bianjibu.
AB The construction and expression of anti-HBsAg and anti-RBC **bispecific** minibody were studied by using anti-HBsAg and anti-RBC **single chain Fv** (ScFv). A "knob" variant T366W was first obtained by replacement of a small acid with a larger one in the human IgG1 CH3 domain. The knob was designed to insert into a "hole" in another CH3 domain which was created by replacement of three large residues with three smaller residues: T366S: L368A: Y407V. The "knob-into-hole" mutation was : S354C: T366W/Y349C: T366S: L368A: Y407V. Then a disulfide bond was engineered in combination with previously designed "knob" or "hole" CH3 was connected to anti- HBsAg or anti-RBC ScFv genes resp. Then the two gene were combined together to form a **bispecific** minibody expression vector. The **bispecific** minibodies were expressed in E.coli. Three different form of anti-HBsAg and anti-RBC minibody expression vectors were constructed. They contained wild-type CH3, "knob-into-hole" CH3 or "knob-into-hole" plus disulfide bond CH3 resp. The results indicated that these three different types of bacterially expressed minibodies had similar HBsAg and RBC binding activities. The second and third type of minibody could cause agglutination of human RBC when HBsAg was present, which demonstrated **bispecific** function. Engineered interface of CH3 could promote formation of heterodimers of different antibodies and facilitate the formation of **bispecific** antibodies (**bispecific** minibody) in E.coli expression system.

L28 ANSWER 16 OF 128 CAPLUS COPYRIGHT 2002 ACS
2001:435822 Document No. 135:136158 A new model for intermediate molecular weight recombinant **bispecific** and trispecific antibodies by efficient heterodimerization of single chain variable domains through fusion to a Fab-chain. Schoonjans, Reinhilde; Willems, An; Schoonooghe, Steve; Leoen, Jannick; Grooten, Johan; Mertens, Nico (Department of Molecular Biology, Molecular Immunology Unit, Flanders Interuniversity, Institute for Biotechnology (VIB), University of Ghent, Ghent, B-9000, Belg.). Biomolecular Engineering, 17(6), 193-202 (English) 2001. CODEN: BIENFV. ISSN: 1389-0344. Publisher: Elsevier Science B.V..
AB Due to their specificity and versatility in use, **bispecific** antibodies (BsAbs) are promising therapeutic tools in tomorrow's medicine, provided sufficient BsAb can be produced. Expression systems favoring efficient heterodimerization of intermediate-sized **bispecific** antibodies will significantly improve existing prodn. methods. Recombinant BsAb can be made by fusing single chain variable fragments (scFv) to a heterodimerization domain. We compare the efficiency of the isolated CL and CH1 const. domains with complete Fab chains to drive

heterodimerization of BaAbs in mammalian cells. We found that the isolated CL:CH1 domain interaction was inefficient for secretion of heterodimers. However, when the complete Fab chains were used, secretion of a heterodimerized **bispecific** antibody was successful. Since the Fab chain encodes a binding specificity on its own, **bispecific** (BsAb) or trispecific (TsAb) antibodies can be made by C-terminal fusion of scFv mols. to the L or Fd Fab chains. This gave rise to disulfide stabilized Fab-scFv BsAb (Bibody)or Fab-(scFv)2 TsAb (Tribody) of intermediate mol. size. Heterodimerization of the L and Fd-contg. fusion proteins was very efficient, and up to 90% of all secreted antibody fragments was in the desired heterodimerized format. All building blocks remained functional in the fusion product, and the **bispecific** character of the mols. as well as the immunol. functionality was demonstrated.

L28 ANSWER 17 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
2001:822167 The Genuine Article (R) Number: 482LX. Dimeric and trimeric antibodies: high avidity scFvs for cancer targeting. Kortt A A (Reprint); Dolezal O; Power B E; Hudson P J. CSIRO, 343 Royal Parade, Parkville, Vic 3052, Australia (Reprint); CSIRO, Parkville, Vic 3052, Australia; CRC Diagnost Technol, Parkville, Vic 3052, Australia. BIOMOLECULAR ENGINEERING (15 OCT 2001) Vol. 18, No. 3, Sp. iss. SI, pp. 95-108. Publisher: ELSEVIER SCIENCE BV. PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 1389-0344. Pub. country: Australia. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Recombinant antibody fragments can be engineered to assemble into stable multimeric oligomers of high binding avidity and specificity to a wide range of target antigens and haptens. This review describes the design and expression of diabodies (dimers), triabodies (trimers) and tetrabodies (tetramers). In particular we discuss the role of linker length between V-domains and the orientation of the V-domains to direct the formation of either diabodies (60 kDa), triabodies (90 kDa) or tetrabodies (120 kDa), and how the size, flexibility and valency of each molecules is suited to different applications for in vivo imaging and therapy. **Single chain Fv** antibody fragments joined by polypeptide linkers of at least 12 residues irrespective of V-domains orientation predominantly form monomers with varying amounts of dimer and higher molecular mass oligomers in equilibrium. A scFv molecule with a linker of 3-12 residues cannot fold into a functional Fv domain and instead associates with a second, scFv molecule to form a bivalent dimer (diabody, similar to 60 kDa). Reducing the linker length below three residues can force scFv association into trimers (triabodies, similar to 90 kDa) or tetramers (similar to 120 kDa) depending on linker length, composition and V-domain orientation. A particular advantage for tumour targeting is that molecules of 60-100 kDa have increased tumour penetration and fast clearance rates compared with the parent Ig (150 kDa). We highlight a number of cancer-targeting scFv diabodies that have undergone successful pre-clinical trials for in vivo stability and efficacy. We also briefly review the design of multi-specific Fv modules suited to cross-link two or more different target antigens. Bi-specific diabodies formed by association of different scFv molecules have been designed as cross-linking reagents for T-cell recruitment into tumours (immunotherapy), viral retargeting (gene therapy) and as red blood cell agglutination reagents (immuno diagnostics). The more challenging trispecific multimers (triabodies) remain to be described.

L28 ANSWER 18 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:273381 Document No.: PREV200100273381. Recombinant **bispecific** single chain antibodies specific against alphaviruses: Development and characterization. Kriangkum, J. (1); Fulton, R. E.; Nagata, L.; Suresh, M. R. (1). (1) Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, T6G 2N8 Canada. Antiviral Research, (April, 2001)

Vol. 50, No. 1, pp. A71. print. Meeting Info.: Fourteenth International Conference on Antiviral Research Seattle, Washington, USA April 08-12, 2001 ISSN: 0166-3542. Language: English. Summary Language: English.

L28 ANSWER 19 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC. DUPLICATE 7

2001:157343 Document No.: PREV200100157343. Design and application of diabodies, triabodies and tetrabodies for cancer targeting. Todorovska, Aneta; Roovers, Rob C.; Dolezal, Olan; Kortt, Alexander A.; Hoogenboom, Hennie R.; Hudson, Peter J. (1). (1) CSIRO Health Science and Nutrition and CRC for Diagnostic Technologies, 343 Royal Parade, Parkville, VIC, 3052: peter.hudson@hsn.csiro.au Australia. Journal of Immunological Methods, (1 February, 2001) Vol. 248, No. 1-2, pp. 47-66. print. ISSN: 0022-1759. Language: English. Summary Language: English.

AB Multivalent recombinant antibody fragments provide high binding avidity and unique specificity to a wide range of target antigens and haptens. This review describes the design and expression of diabodies, triabodies and tetrabodies using examples of scFv molecules that target viruses (influenza neuraminidase) and cancer (Ep-CAM; epithelial cell adhesion molecule). We discuss the preferred choice of linker length between V-domains to direct the formation of either diabodies (60 kDa), triabodies (90 kDa) or tetrabodies (120 kDa), each with size, flexibility and valency suited to different applications for in vivo imaging and therapy. The increased binding valency of these scFv multimers results in high avidity (low off-rates). A particular advantage for tumour targeting is that molecules of 60-100 kDa have increased tumour penetration and fast clearance rates compared to the parent Ig (150 kDa). We highlight a number of cancer-targeting scFv multimers that have recently successfully undergone pre-clinical trials for in vivo stability and efficacy. We also review the design of multi-specific Fv modules suited to cross-link two or more different target antigens. These bi- and tri-specific multimers can be formed by association of different scFv molecules and, in the first examples, have been designed as cross-linking reagents for T-cell recruitment into tumours (immunotherapy), viral retargeting (gene therapy) and as red blood cell agglutination reagents (immunodiagnostics).

L28 ANSWER 20 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)

2001:70178 The Genuine Article (R) Number: 388WE. Antibody constructs for radioimmunodiagnosis and treatment of human pancreatic cancer. Goel A; Batra S K (Reprint). Univ Nebraska, Med Ctr, Eppley Inst Res Canc & Allied Dis, Dept Biochem & Mol Biol, 984525 Nebraska Med Ctr, Omaha, NE 68198 USA (Reprint); Univ Nebraska, Med Ctr, Eppley Inst Res Canc & Allied Dis, Dept Biochem & Mol Biol, Omaha, NE 68198 USA. TERATOGENESIS CARCINOGENESIS AND MUTAGENESIS (FAL 2001) Vol. 21, No. 1, pp. 45-57. Publisher: WILEY-LISS. DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012 USA. ISSN: 0270-3211. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Pancreatic cancer (PC) is a common disease that is seldom cured. Current approaches to the treatment of PC are not effective because the non-specific nature of both chemotherapy and external beam radiation results in toxicity to normal tissue. Monoclonal antibodies (MAbs) can be used as selective carriers for delivering radionuclides, toxins, or cytotoxic drugs to malignant cell populations. Therefore, MAb-technology has led to a significant amount of research in targeted therapy. Targeted therapy would generally allow the concentration of cytotoxic agents in tumors and would markedly lessen the toxicity to normal tissues, which limits the dosage and effectiveness of systemically administered drugs. A variety of MAbs are being pre-clinically evaluated for the diagnosis and treatment of PC. Novel recombinant antibody constructs hold a promising future in both the diagnosis and treatment of cancer. By genetic-engineering methods, several high affinity antibody fragments with optimum tumor targeting properties, such as higher functional affinity (divalent and multivalent scFvs) and blood residence time (good tumor

localization with high radiolocalization index), have been generated. Animal models have permitted the in vivo assessment of these antibody-based reagents, therapeutic/diagnostic radionuclide, radiolabeling conditions, and efficacy of administration regimes. For PC, immunoscintigraphy using MAbs has taken new strides. The use of MAbs and their fragments for radioimmunoguided surgery and therapy of PC has shown encouraging results at preclinical levels and warrants further attention. Teratogenesis Carcinog. Mutagen. 21:45-57, 2001. (C) 2001 Wiley-Liss, Inc.

L28 ANSWER 21 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:152795 Document No.: PREV200100152795. Ribosome display and affinity maturation: From antibodies to single V-domains and steps towards cancer therapeutics. Irving, Robert A. (1); Coia, Gregory; Roberts, Anthony; Nuttall, Stewart D.; Hudson, Peter J.. (1) CSIRO Health Sciences and Nutrition and CRC for Diagnostic Technologies, 343 Royal Parade, Parkville, VIC, 3052: bob.irving@hsn.csiro.au Australia. Journal of Immunological Methods, (1 February, 2001) Vol. 248, No. 1-2, pp. 31-45. print. ISSN: 0022-1759. Language: English. Summary Language: English.
AB Protein affinity maturation using molecular evolution techniques to produce high-affinity binding proteins is an important step in the generation of reagents for cancer diagnosis and treatment. Currently, the most commonly used molecular evolution processes involve mutation of a single gene into complex gene repertoires followed by selection from a display library. Fd-bacteriophage are the most popular display vectors, but are limited in their capacity for library presentation, speed of processing and mutation frequency. Recently, the potential of ribosome display for directed molecular evolution was recognised and developed into a rapid and simple affinity selection strategy using ribosome complexes to display antibody fragments (scFv). Ribosome display and selection has the potential to generate and display large libraries more representative of the theoretical optima for naive repertoires (1014). Even more important is the application of ribosome display for the affinity maturation of individual proteins by rapid mutation and selection cycles. These display strategies can apply to other members of the immunoglobulin superfamily; for example single V-domains which have an important application in providing specific targeting to either novel or refractory cancer markers. We discuss the application of ribosome display and selection in conjunction with variable domain (CTLA-4) libraries as the first step towards this objective and review affinity maturation strategies for in vitro ribosome display systems.

L28 ANSWER 22 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
2001:104504 The Genuine Article (R) Number: 394AE. Engineering and characterization of a novel fusion protein incorporating B7.2 and an anti-ErbB-2 single-chain antibody fragment for the activation of jurkat T cells. Marshall K W; Marks J D (Reprint). San Francisco Gen Hosp, 1001 Potrero Ave, Room 3C-38, San Francisco, CA 94110 USA (Reprint); Univ Calif San Francisco, Dept Anesthesia, San Francisco, CA 94143 USA; Univ Calif San Francisco, Dept Pharmaceut Chem, San Francisco, CA 94143 USA. JOURNAL OF IMMUNOTHERAPY (JAN-FEB 2001) Vol. 24, No. 1, pp. 27-36. Publisher: LIPPINCOTT WILLIAMS & WILKINS. 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. ISSN: 1053-8550. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB The provision of the T-cell costimulatory molecule B7 to tumor cells can be an effective way to trigger a tumor-specific cytolytic T-cell response. One way to provide B7 to tumor cells would be to couple an antitumor antibody directly to B7. Such a molecule should target tumors displaying antigen and provide the costimulatory signal to T cells, resulting in the initiation of an antitumor T-cell response. To this end, a fusion protein was designed that incorporates a single-chain antibody fragment (scFv) to erbB-2 (Her2/neu), an oncogene product overexpressed by 30% to 50% of breast carcinomas, and the ECD of B7-2 (CD86). This fusion protein, expressed and purified from Pichia pastoris, was shown to retain

binding activity to both counter receptors, erbB-2 and CD28. The fusion protein was also shown to target erb-2-positive tumor cells and to deliver a CD28-specific T-cell costimulatory signal. These results suggest that a fusion protein engineered to target tumor cells and signal T cells for activation may be an effective means of cancer immunotherapy. Further studies should be performed to characterize the fusion protein in erbB-2 tumor-bearing mice for in vivo tumor targeting, biodistribution, and efficacy.

L28 ANSWER 23 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
2001:824578 The Genuine Article (R) Number: 481FH. A review of modifications to recombinant antibodies: attempt to increase efficacy in oncology applications. Reff M E (Reprint); Heard C. Idec Pharmaceut Corp, 3010 Sci Pk Rd, POB 919080, San Diego, CA 92191 USA (Reprint); Idec Pharmaceut Corp, San Diego, CA 92191 USA. CRITICAL REVIEWS IN ONCOLOGY HEMATOLOGY (OCT 2001) Vol. 40, No. 1, pp. 25-35. Publisher: ELSEVIER SCIENCE INC. 655 AVENUE OF THE AMERICAS, NEW YORK, NY 10010 USA. ISSN: 1040-8428. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Although monoclonal antibodies have high specificity, their usefulness in the clinic, especially against solid tumors, has been limited. This arises in part from the inability of antibody molecules to penetrate into the tumor and kill the tumor cells. In addition, natural cytotoxic effects of antibodies, mediated through complement or Fc receptors, may not be sufficient to kill malignant cells. This review will present some of the antibody modifications used to increase efficacy. Modified recombinant antibodies have been designed to be more cytotoxic (immunotoxins), to increase natural effector functions (bivalent antibodies, antibody-fusion molecules, multimeric antibodies, directed mutations in Fc region), or to pretarget cells for concentration of cytotoxic drugs. This review will also focus on engineering of smaller versions of antibodies that retain specificity (**single chain Fvs**, Fabs, Fab(2)s. minibodies. domain deleted antibodies) and have increased penetrability of solid tumors. Many of these antibody modifications may result in antigenic compounds which can limit repeat administration, Clinical experiences will be highlighted if information is available.

L28 ANSWER 24 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:157342 Document No.: PREV200100157342. Selection of cell binding and internalizing epidermal growth factor receptor antibodies from a phage display library. Heitner, Tara; Moor, Anne; Garrison, Jennifer L.; Marks, Cara; Hasan, Tayyaba; Marks, James D. (1). (1) Departments of Anesthesia and Pharmaceutical Chemistry, University of California, San Francisco, San Francisco General Hospital, 1001 Potrero Avenue, Room 3C-38, San Francisco, CA, 94110: marksj@anesthesia.ucsf.edu USA. Journal of Immunological Methods, (1 February, 2001) Vol. 248, No. 1-2, pp. 17-30. print. ISSN: 0022-1759. Language: English. Summary Language: English.
AB The first step in developing a targeted cancer therapeutic is generating a ligand that binds to a receptor which is either tumor specific or sufficiently overexpressed in tumors to provide targeting specificity. For this work, we generated human monoclonal antibodies to the EGF receptor (EGFR), an antigen overexpressed on many solid tumors. **Single chain Fv** (scFv) antibody fragments were directly selected by panning a phage display library on tumor cells (A431) overexpressing EGFR or Chinese hamster ovary cells (CHO/EGFR cells) transfected with the EGFR gene and recovering endocytosed phage from within the cell. Three unique scFvs were isolated, two from selections on A431 cells and two from selections on CHO/EGFR cells. All three scFv bound native receptor as expressed on a panel of tumor cells and did not bind EGFR negative cells. Phage antibodies and multivalent immunoliposomes constructed from scFv were endocytosed by EGFR expressing cells as shown by confocal microscopy. Native scFv primarily stained the cell surface,

with less staining intracellularly. The results demonstrate how phage antibodies binding native cell surface receptors can be directly selected on overexpressing cell lines or transfected cells. Use of a transfected cell line allows selection of antibodies to native receptors without the need for protein expression and purification, significantly speeding the generation of targeting antibodies to genomic sequences. Depending upon the format used, the antibodies can be used to deliver molecules to the cell surface or intracellularly.

L28 ANSWER 25 OF 128 MEDLINE DUPLICATE 8
2001316759 Document Number: 21283404. PubMed ID: 11388794. Expression and purification of monospecific and **bispecific** recombinant antibody fragments derived from antibodies that block the CD80/CD86-CD28 costimulatory pathway. Dincq S; Bosman F; Buyse M A; Degrieck R; Celis L; de Boer M; Van Doorsselaere V; Sablon E. (Department of Microbiology, Innogenetics NV, Industriepark Zwijnaarde 7, Box 4, B-9052 Gent, Belgium.. stephanie_dincq@innogenetics.be) . PROTEIN EXPRESSION AND PURIFICATION, (2001 Jun) 22 (1) 11-24. Journal code: 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.

AB The development of recombinant techniques for rapid cloning, expression, and characterization of cDNAs encoding antibody (Ab) subunits has revolutionized the field of antibody engineering. By fusion to heterologous protein domains, chain shuffling, or inclusion of self-assembly motifs, novel molecules such as **bispecific** Abs can be generated that possess the subset of functional properties designed to fit the intended application. We describe the engineering of Ab fragments produced in bacteria for blocking the CD28-CD80/CD86 costimulatory interaction in order to induce tolerance against transplanted organs. We designed **single-chain Fv** antibodies, monospecific and **bispecific** diabodies, and a **bispecific** tetravalent antibody (BiTAb) molecule directed against the CD80 and/or CD86 costimulatory molecules. These recombinant Ab molecules were expressed in Escherichia coli, followed by purification and evaluation for specific interaction with their respective antigen in an enzyme-linked immunosorbent assay (ELISA). A specific sandwich ELISA confirmed the bispecificity of the **bispecific** diabodies and the BiTAb. Copyright 2001 Academic Press.

L28 ANSWER 26 OF 128 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
2001075269 EMBASE Introduction: **Bispecific** antibodies. Segal D.M.; Weiner G.J.; Weiner L.M.. D.M. Segal, Experimental Immunology Branch, National Cancer Institute, NIH, Bethesda, MD 20892-1360, United States. Journal of Immunological Methods 248/1-2 (1-6) 1 Feb 2001. Refs: 16. ISSN: 0022-1759. CODEN: JIMMBG. Publisher Ident.: S 0022-1759(00)00338-0. Pub. Country: Netherlands. Language: English.

L28 ANSWER 27 OF 128 CAPLUS COPYRIGHT 2002 ACS
2000:666777 Document No. 133:251277 A novel chimeric protein for prevention and treatment of HIV infection. Berger, Edward A.; Del Castillo, Christie M. (United States of America, Department of Health & Human Services, the Nat, USA). PCT Int. Appl. WO 2000055207 A1 20000921, 55 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US6946 20000316. PRIORITY: US 1999-PV124681 19990316.

AB This invention relates to **bispecific** fusion proteins effective

in viral neutralization. More specifically, such proteins have two different binding domains, an inducing-binding domain and an induced-binding domain, functionally linked by a peptide linker. Such proteins, nucleic acid mols. encoding them, and their prodn. and use in preventing or treating viral infections are provided. One prototypical **bispecific** fusion protein is sCD4-scFv(17b), in which a sol. CD4 fragment (contg. domains D1 and D2) is fused to a **single chain Fv** portion of antibody 17b via a linker.

L28 ANSWER 28 OF 128 MEDLINE

2001062947 Document Number: 20552173. PubMed ID: 11103810. Targeting of bivalent anti-ErbB2 diabody antibody fragments to tumor cells is independent of the intrinsic antibody affinity. Nielsen U B; Adams G P; Weiner L M; Marks J D. (Department of Anesthesiology and Pharmaceutical Chemistry, University of California, San Francisco 94110, USA.) CANCER RESEARCH, (2000 Nov 15) 60 (22) 6434-40. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB In immunodeficient mice antitumor **single-chain Fv** (scFv) molecules penetrate tumors rapidly and have rapid serum clearance, leading to excellent tumor:normal organ ratios. However, the absolute quantity of scFv retained in the tumor is low due to rapid serum clearance and monovalent scFv binding. We previously demonstrated that the presence of an additional binding site prolongs in vitro and in vivo association of scFv-based molecules with tumor cells expressing relevant antigen. The contribution of the intrinsic affinity of each component scFv to the association between a dimeric scFv and its target antigen is largely unknown. Here, we have constructed bivalent diabody molecules from three affinity mutants of the human anti-ErbB2 (HER2/neu) scFv molecule C6.5 by shortening the peptide linker between the heavy (VH) and light (VL) chains variable domains from 15 to 5 amino acids. The shorter linker prevents intramolecular pairing of VH and VL, resulting in intermolecular pairing and creation of a dimeric Mr 50,000 molecule with two antigen-binding sites. The scFv used to create the diabodies span a 133-fold range of affinity for the same epitope of ErbB2 [133 nM (C6G98A), 25 nM (C6.5), and 1 nM (C6ML3-9)] and differ by only one to three amino acids. Diabody binding kinetics were determined by surface plasmon resonance on the immobilized ErbB2 extracellular domain. The association rate constants obtained for each diabody molecule were similar to that of the parental (component) scFv. However, the dissociation rate constants obtained for the bivalent diabodies were up to 15-fold slower. The magnitude of the decrease in the bivalent dissociation rate constant was inversely proportional to the monovalent interaction, ranging from only 3-fold for that of the C6ML3-9 diabody to 15-fold for the C6G98A diabody. This resulted in only a 22-fold difference in bivalent affinity, compared with a 133-fold difference in affinity for the respective scFv. Equilibrium-binding constants obtained by surface plasmon resonance correlated well with the equilibrium-binding constants determined in vitro on ErbB2 overexpressing cells. Biodistribution studies were performed in scid mice bearing established SKOV3 tumors. At 24 h, 3-37-fold more diabody was retained in tumor compared with the parental scFv monomers. This likely results from a higher apparent affinity, because of bivalent binding, and a slower serum clearance. Surprisingly, the differences in affinity between diabodies did not result in differences in quantitative tumor retention or tumor to blood ratios. In fact, the diabody constructed from the lowest affinity scFv exhibited the best tumor-targeting properties. We conclude that, above a threshold affinity, other factors regulate quantitative tumor retention. In addition, straightforward dimerization of a low-affinity scFv leads to significantly greater tumor localization than does exhaustive scFv affinity maturation.

L28 ANSWER 29 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)

2001:44389 The Genuine Article (R) Number: 387KH. New approaches to antibody therapy. Weiner L M (Reprint); Adams G P. Fox Chase Canc Ctr, Dept Med

Oncol, 7701 Burholme Ave, Philadelphia, PA 19111 USA (Reprint); Fox Chase Canc Ctr, Dept Med Oncol, Philadelphia, PA 19111 USA. ONCOGENE (11 DEC 2000) Vol. 19, No. 53, pp. 6144-6151. Publisher: NATURE PUBLISHING GROUP. HOUNDMILLS, BASINGSTOKE RG21 6XS, HAMPSHIRE, ENGLAND. ISSN: 0950-9232. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Antibody-based therapy of human cancers has led to several remarkable outcomes, particularly in the therapy of breast cancer and lymphoma. Many solid tumors have proven less responsive, due in part to difficulties in the tumor-selective delivery of antibodies and potential cytolytic effecters. However, antibodies that directly perturb signaling mechanisms in cells derived from epithelial malignancies have shown benefit; examples include antibodies directed against the extracellular domains of HER2/neu and epidermal growth factor receptor. A long-term goal of immunotherapy has been to induce anti-tumor inflammatory responses that can directly cause tumor regression or induct adaptive responses against tumor-related antigens. This review focuses on the use of antibodies to provide a means for initiating anti-tumor immune responses, and on the use of antibodies as delivery vehicles of radionuclides.

L28 ANSWER 30 OF 128 MEDLINE DUPLICATE 9

2000173579 Document Number: 20173579. PubMed ID: 10706880. A recombinant **bispecific** single-chain antibody, CD19 x CD3, induces rapid and high lymphoma-directed cytotoxicity by unstimulated T lymphocytes. Loffler A; Kufer P; Lutterbuse R; Zettl F; Daniel P T; Schwenkenbecher J M; Riethmuller G; Dorken B; Bargou R C. (Max Delbruck Center for Molecular Medicine, Berlin-Buch, Germany.) BLOOD, (2000 Mar 15) 95 (6) 2098-103. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Although **bispecific** antibodies directed against malignant lymphoma have been shown to be effective in vitro and in vivo, extended clinical trials so far have been hampered by the fact that conventional approaches to produce these antibodies suffer from low yields, ill-defined byproducts, or laborious purification procedures. To overcome this problem, we have generated a small, recombinant, lymphoma-directed, **bispecific** single-chain (bsc) antibody according to a novel technique recently described. The antibody consists of 2 different **single-chain Fv** fragments joined by a glycine-serine linker. One specificity is directed against the CD3 antigen of human T cells, and the other antigen-binding site engages the pan-B-cell marker CD19, uniformly expressed on the vast majority of B-cell malignancies. The construct was expressed in Chinese hamster ovary cells and purified by its C-terminal histioline tag. Specific binding to CD19 and CD3 was demonstrated by fluorescence-activated cell sorter analysis. By redirecting unstimulated primary human T cells derived from the peripheral blood against CD19-positive lymphoma cells, the bscCD19 x CD3 antibody showed significant cytotoxic activity at very low concentrations of 10 to 100 pg/mL and at effector to target cell ratios as low as 2:1. Moreover, strong lymphoma-directed cytotoxicity at low antibody concentrations was rapidly induced during 4 hours even in experiments without any T-cell prestimulation. Thus, this particular antibody proves to be much more efficacious than the **bispecific** antibodies described until now. Therefore, the described bscCD19 x CD3 molecule should be a suitable candidate to prove the therapeutic benefit of **bispecific** antibodies in the treatment of non-Hodgkin lymphoma. (Blood. 2000;95:2098-2103)

L28 ANSWER 31 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)

2000:702250 The Genuine Article (R) Number: 352MK. Construction and in vivo evaluation of an anti-PSA x anti-CD3 **bispecific** antibody for the immunotherapy of prostate cancer. Katzenwadel A; Schleer H; Gierschner D; Wetterauer U; ElsasserBeile U (Reprint). UNIV FREIBURG, DEPT UROL, EXPT RES GRP, STEFAN MEIER STR 8, D-79106 FREIBURG, GERMANY (Reprint); UNIV

FREIBURG, DEPT UROL, EXPT RES GRP, D-79106 FREIBURG, GERMANY. ANTICANCER RESEARCH (MAY-JUN 2000) Vol. 20, No. 3A, pp. 1551-1555. Publisher: INT INST ANTICANCER RESEARCH. EDITORIAL OFFICE 1ST KM KAPANDNTIOU-KALAMOU RD KAPANDRITI, POB 22, ATHENS 19014, GREECE. ISSN: 0250-7005. Pub. country: GERMANY. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: Cross-linking of tumor antigens with the T-cell associated CD3 antigen can be effectively achieved by **bispecific** monoclonal antibodies and lead to an increase in antigen-specific cytotoxicity in T cells. Because of the high organ specificity of the prostate specific antigen (PSA) a **bispecific** antibody (BiAb) directed against this antigen and CD3 may be a tool for a highly specific immune therapy of prostate cancer. Methods: For generating BiAb, the quadroma technique was used. Binding properties both to CD3 and PSA were shown by flow cytometry with the CD3 expressing Jurkat cell line and fluorescein-labeled PSA. Specific tumor cell lysis was tested with the PSA expressing prostate carcinoma cell line LNCaP as target and interleukin-2 activated human peripheral blood lymphocytes as effector cells in a chromium-51-release assay. For in vivo evaluation of the BiAb, a nude mouse model was used. The mice were inoculated with LNCaP prostate carcinoma cells. Animals with growing tumors were treated with 100 mu g BiAb and 5x10(6) effector cells. Results: Three stable quadromas producing anti-CD3 x anti-PSA BiAb were established From the culture supernatant of one quadroma, BiAb was separated by affinity chromatography and rested in vitro and in vivo for its ability to target effector T lymphocytes against appropriate tumor cells. In vitro, a specific lysis of PSA expressing prostate carcinoma cells was demonstrated In vivo a significant reduction in tumor growth ($p < 0.05$) could be shown in nude mice treated with BiAb and effector cells as compared to a group treated only with effector cells and an untreated control group. Conclusion: In the present study, an anti-CD3 x anti-PSA-BiAb was demonstrated to be effective against prostate carcinoma cells in vitro and in vivo. Therefore this BiAb may be a tool for the immunotherapy of prostate cancer.

L28 ANSWER 32 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
2000:631555 The Genuine Article (R) Number: 344VT. Recombinant antibodies: a novel approach to cancer diagnosis and therapy. Hudson P J (Reprint). CSIRO, CRC DIAGNOST TECHNOL, 343 ROYAL PARADE, PARKVILLE, VIC 3052, AUSTRALIA (Reprint). EXPERT OPINION ON INVESTIGATIONAL DRUGS (JUN 2000) Vol. 9, No. 6, pp. 1231-1242. Publisher: ASHLEY PUBL LTD. 1ST FL, THE LIBRARY, 1 SHEPHERDS HILL HIGHGATE, LONDON N6 5QJ, ENGLAND. ISSN: 1354-3784. Pub. country: AUSTRALIA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Recombinant antibodies and their fragments currently represent over 30% of all biological proteins undergoing clinical trials for diagnosis and therapy. These reagents dominate the cancer-targeting field, as highlighted by the recent approval of the first engineered therapeutic antibodies by the Food and Drugs Administration (FDA). Last year, important advances have been made in the design, selection and production of recombinant antibodies. The natural immune repertoire and somatic cell affinity maturation has been superseded by large antibody display libraries and rapid molecular evolution strategies. These novel libraries and selection methods have enabled the rapid isolation of high-affinity cancer targeting and antiviral antibodies, the latter capable of redirecting viruses for gene therapy applications. In alternative strategies for cancer diagnosis and therapy, recombinant antibody fragments have been fused to radioisotopes, drugs, toxins, enzymes and biosensor surfaces. Antibody-directed cancer pre-targeting followed by prodrug activation (ADEPT) has proved a most promising therapeutic strategy. Multi-specific antibodies have been effective for cytotoxic T-cell recruitment and antibody-fusion proteins have delivered enhanced immunotherapeutic and vaccination strategies. The new millennium is indeed an exciting time for the design, selection and formulation of a range of

new antibody-based products for cancer diagnosis and therapy.

L28 ANSWER 33 OF 128 MEDLINE DUPLICATE 10
2001400716 Document Number: 21345211. PubMed ID: 11451417. Generation and characterization of a novel tetravalent **bispecific** antibody that binds to hepatitis B virus surface antigens. Park S S; Ryu C J; Kang Y J; Kashmiri S V; Hong H J. (The Antibody Engineering Research Laboratory, Korea Research Institute of Bioscience and Biotechnology, PO Box 115, Yuseong, Taejon 305-600, South Korea.) MOLECULAR IMMUNOLOGY, (2000 Dec) 37 (18) 1123-30. Journal code: 7905289. ISSN: 0161-5890. Pub. country: England: United Kingdom. Language: English.

AB Hepatitis B virus (HBV) infection is a worldwide public health problem affecting about 350 million people. HBV envelope contains three surface antigens, called pre-S1, pre-S2 and S. For the prophylaxis of HBV infection, only an anti-S monoclonal antibody was tested for the protective efficacy against HBV infection, but it was shown to be incomplete. In addition, some immune escape mutants carrying mutations on the S antigen were reported. Therefore, a multivalent **bispecific** antibody rather than a single monoclonal antibody would be more beneficial for the prophylaxis of HBV infection. We have generated a novel tetravalent **bispecific** antibody with two binding sites for each of the S and pre-S2 antigens. Each of the antigen-binding sites was composed of a **single-chain Fv** (ScFv). The tetravalent antibody was generated by constructing a single gene encoding a single-chain protein. This protein consisted of an anti-S ScFv whose carboxyl end was tethered, through a 45 amino acid linker, to the amino terminus of anti-preS2 ScFv that in turn was joined to the hinge region of human gamma1 constant region. The single-chain protein was expressed in Chinese hamster ovary cells and secreted in culture supernatant as a homodimeric molecule. The tetravalent **bispecific** antibody showed both anti-S and anti-pre-S2 binding activities. In addition, the binding affinity of the **bispecific** antiboy for HBV particles was greater than that of either parental antibody. The tetravalent **bispecific** antibody is a potentially useful reagent for the prevention and treatment of HBV infection.

L28 ANSWER 34 OF 128 MEDLINE DUPLICATE 11
2000493637 Document Number: 20336368. PubMed ID: 10880021. Targeting of adenoviral vectors through a **bispecific** single-chain antibody. Haisma H J; Grill J; Curiel D T; Hoogeland S; van Beusechem V W; Pinedo H M; Gerritsen W R. (Department of Medical Oncology, University Hospital Vrije Universiteit, Amsterdam, The Netherlands.. hj.haisma@azvu.nl) . CANCER GENE THERAPY, (2000 Jun) 7 (6) 901-4. Journal code: 9432230. ISSN: 0929-1903. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Recombinant adenoviral vectors are attractive in the context of cancer gene therapy because they are capable of delivering genes to a wide variety of tissues. The utility of adenoviruses is limited by their lack of specificity and by the absence of the receptor(s) for these viruses on many tumor cells. Redirecting adenoviral vectors to tissue- or tumor-specific targets can be achieved by using **bispecific** conjugates produced by chemical linkage of an anti-adenovirus antibody (Ab) and a ligand or Ab directed toward a specific target. To avoid the limitations of chemical conjugates, molecular conjugates of anti-fiber knob and ligand have been proposed. We present here a novel strategy that allows the production of recombinant **bispecific** single-chain Abs directed at cell surface molecules. A construct was made that encodes a neutralizing anti-adenovirus fiber **single-chain Fv** (scFv) Ab (S11) fused to a scFv Ab (425) directed against the epidermal growth factor receptor. The fusion protein markedly enhanced the infection efficiency of adenoviral vectors in epidermal growth factor receptor-expressing cell lines. The **bispecific** scFv could be purified and concentrated after binding of its 6His tag to a nickel column without significant loss of activity. This approach should permit the

production of high quantities of active **bispecific** scFv for in vivo use. The universal design of the construct allows rapid screening for relevant specific scFv directed at cell surface antigens that can be incorporated into adenoviral targeting strategies.

L28 ANSWER 35 OF 128 MEDLINE DUPLICATE 12
2000341691 Document Number: 20341691. PubMed ID: 10878363. Treatment of human B cell lymphoma xenografts with a CD3 x CD19 diabody and T cells. Cochlovius B; Kipriyanov S M; Stassar M J; Christ O; Schuhmacher J; Strauss G; Moldenhauer G; Little M. (Recombinant Antibody Research Group, Department of Tumor Progression and Immune Defense, German Cancer Research Center (DKFZ), Heidelberg, Germany.) JOURNAL OF IMMUNOLOGY, (2000 Jul 15) 165 (2) 888-95. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.
AB The use of anti-CD3 x antitumor **bispecific** Abs is an attractive and highly specific approach in cancer therapy. Recombinant Ab technology now provides powerful tools to enhance the potency of such immunotherapeutic constructs. We designed a heterodimeric diabody specific for human CD19 on B cells and CD3epsilon chain of the TCR complex. After production in Escherichia coli and purification, we analyzed its affinity, stability, and pharmacokinetics, and tested its capacity to stimulate T cell proliferation and mediate in vitro lysis of CD19+ tumor cells. The effect of the diabody on tumor growth was investigated in an in vivo model using immunodeficient mice bearing a human B cell lymphoma. The CD3 x CD19 diabody specifically interacted with both CD3- and CD19-positive cells, was able to stimulate T cell proliferation in the presence of tumor cells, and induced the lysis of CD19+ cells in the presence of activated human PBL. The lytic potential of the diabody was enhanced in the presence of an anti-CD28 mAb. In vivo experiments indicated a higher stability and longer blood retention of diabodies compared with **single chain Fv** fragments. Treatment of immunodeficient mice bearing B lymphoma xenografts with the diabody and preactivated human PBL efficiently inhibited tumor growth. The survival time was further prolonged by including the anti-CD28 mAb. The CD3 x CD19 diabody is a powerful tool that should facilitate the immunotherapy of minimal residual disease in patients with B cell leukemias and malignant lymphomas.

L28 ANSWER 36 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
2001:26550 The Genuine Article (R) Number: 385GM. Expression of a **bispecific** dsFv-dsFv ' antibody fragment in Escherichia coli. Schmiedl A; Breitling F; Dubel S (Reprint). Univ Heidelberg, Inst Genet Mol, Neuenheimer Feld 203, D-69120 Heidelberg, Germany (Reprint); Univ Heidelberg, Inst Genet Mol, D-69120 Heidelberg, Germany; German Canc Res Ctr, D-69120 Heidelberg, Germany. PROTEIN ENGINEERING (OCT 2000) Vol. 13, No. 10, pp. 725-734. Publisher: OXFORD UNIV PRESS. GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND. ISSN: 0269-2139. Pub. country: Germany. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB A **bispecific** disulfide-stabilized Fv antibody fragment (dsFv-dsFv') consisting of two different disulfide-stabilized Fv antibody fragments connected by flexible linker peptides was produced by secretion of three polypeptide chains into the periplasm of Escherichia coli. The dsFv-dsFv' molecules were enriched by immobilized metal affinity chromatography and further purified by anion-exchange chromatography. The recombinant antibody constructs retained the two parental antigen binding specificities and were able to cross-link the two different antigens. The described dsFv-dsFv' design might be of particular value for therapeutic in vivo applications since improved stability is expected to be combined with minimal immunogenicity.

L28 ANSWER 37 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
2000:628014 The Genuine Article (R) Number: 343BB. Natural and designer binding sites made by phage display technology. Hoogenboom H R (Reprint);

Chames P. TARGET QUEST BV, POB 5800, NL-6202 AZ MAASTRICHT, NETHERLANDS (Reprint); MAASTRICHT UNIV, DEPT PATHOL, NL-6202 AZ MAASTRICHT, NETHERLANDS; UNIV HOSP MAASTRICHT, NL-6202 AZ MAASTRICHT, NETHERLANDS. IMMUNOLOGY TODAY (AUG 2000) Vol. 21, No. 8, pp. 371-378. Publisher: ELSEVIER SCI LTD. THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, OXON, ENGLAND. ISSN: 0167-5699. Pub. country: NETHERLANDS. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In the past decade, the drive to develop completely human antibodies for human therapy has led to the development of phage display technology. This technology is able to deliver the ultimate in antibody engineering, that is, high-affinity fully human antibodies to any antigen of choice. Here, this application of phage display technology is reviewed, and the many other antibody-engineering avenues this technology offers are highlighted.

L28 ANSWER 38 OF 128 MEDLINE DUPLICATE 13

2000295268 Document Number: 20295268. PubMed ID: 10835110. An efficient route to the production of an IgG-like **bispecific** antibody. Zuo Z; Jimenez X; Witte L; Zhu Z. (Department of Molecular and Cell Biology, ImClone Systems Incorporated, 180 Varick Street, New York, NY 10014, USA.) PROTEIN ENGINEERING, (2000 May) 13 (5) 361-7. Journal code: 8801484. ISSN: 0269-2139. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Production of IgG-form **bispecific** antibody (BsAb-IgG) by co-expressing two antibodies in transfected cells is often inefficient owing to the unwanted pairing between the component heavy and light chains. We have developed an efficient method for the production of a novel IgG-like BsAb by using the natural dimerization mechanism between IgG heavy and light chains. Two **single-chain Fv** (scFv) of different specificity are fused to the constant domain of human kappa chain (C(L)) and the first constant domain of human heavy chain (C(H1)), to form two polypeptides, (scFv)(1)-C(L) and (scFv)(2)-C(H1)-C(H2)-C(H3), respectively. Co-expression of the two polypeptides in mammalian cells results in the formation of a covalently linked IgG-like hetero-tetramer, Bs(scFv)(4)-IgG, with dual specificity. Our approach yields a homogeneous **bispecific** IgG-like antibody product with each molecule containing four antigen binding sites, two for each of its target antigens. A Bs(scFv)(4)-IgG was prepared using two scFv antibodies each directed against a different epitope of a vascular endothelial growth factor receptor, the kinase insert domain-containing receptor (KDR). The Bs(scFv)(4)-IgG is capable of simultaneously binding to the two epitopes on the receptor. Further, the Bs(scFv)(4)-IgG also retains the antigen-binding efficacy and biological activity of its component antibodies.

L28 ANSWER 39 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)

2000:596175 The Genuine Article (R) Number: 339GX. Antibody phage display applications for nuclear medicine imaging and therapy. Winthrop M D (Reprint); Denardo G L; Denardo S J. UNIV CALIF DAVIS, MED CTR, DEPT INTERNAL MED, DIV RADIODIAG & THERAPY, 1508 ALHAMBRA BLVD, RM 3100, SACRAMENTO, CA 95816 (Reprint). QUARTERLY JOURNAL OF NUCLEAR MEDICINE (SEP 2000) Vol. 44, No. 3, pp. 284-295. Publisher: EDIZIONI MINERVA MEDICA. CORSO BRAMANTE 83-85 INT JOURNALS DEPT., 10126 TURIN, ITALY. ISSN: 0392-0208. Pub. country: USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Antibody-based constructs genetically engineered from genes of diverse origin provide a remarkable opportunity to develop functional molecular imaging techniques and specific molecular targeted radionuclide therapies. Phage display libraries of antibody fragment genes can be used to select antibody-based constructs that bind any chosen epitope. A large naive human antibody-based library was used to illustrate binding of antibody constructs to a variety of common and unique antigens. Antibody-based libraries from hybridoma cells, lymphocytes from immunized humans or from

mice and human antibody repertoires produced in transgenic mice have also been described. Several orders of magnitude of affinity enhancement can be achieved by random or site specific mutations of the selected binding peptide domains of the scFv. Affinities (K-d) as high as 10(-11) M (10 pM) for affinity-matured scFv have been documented. Such gene libraries thus offer an almost limitless variety of antibody-based molecular binding peptide modules that can be used in creative ways for the construction of new targeting agents for functional or molecular imaging and therapy.

L28 ANSWER 40 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
2000:596174 The Genuine Article (R) Number: 339GX. Designer genes: recombinant antibody fragments for biological imaging. Wu A M (Reprint); Yazaki P J. CITY HOPE NATL MED CTR, BECKMAN RES INST, DEPT MOL BIOL, 1450 E DUARTE RD, DUARTE, CA 91010 (Reprint). QUARTERLY JOURNAL OF NUCLEAR MEDICINE (SEP 2000) Vol. 44, No. 3, pp. 268-283. Publisher: EDIZIONI MINERVA MEDICA. CORSO BRAMANTE 83-85 INT JOURNALS DEPT., 10126 TURIN, ITALY. ISSN: 0392-0208. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Monoclonal antibodies (MAbs), with high specificy and high affinity for their target antigens, can be utilized for delivery of agents such as radionuclides, enzymes, drugs, or toxins in vivo. However, the implementation of radio-labeled antibodies as ''magic bullets'' for detection and treatment of diseases such as cancer has required addressing several shortcomings of murine MAbs. These include their immunogenicity, sub-optimal targeting and pharmacokinetic properties, and practical issues of production and radiolabeling. Genetic engineering provides a powerful approach for redesigning antibodies for use in oncologic applications in vivo. Recombinant fragments have been produced that retain high affinity for target antigens, and display a combination of rapid, high-level tumor targeting with concomitant clearance from normal tissues and the circulation in animal models. An important first step was cloning and engineering of antibody heavy and light chain variable domains into **single-chain Fvs** (molecular weight, 25-27 kDa), in which the variable regions are joined via a synthetic Linker peptide sequence. Although scFvs themselves showed limited tumor uptake in preclinical and clinical studies, they provide a useful building block for intermediate-sized recombinant fragments. Covalently linked dimers or non-covalent dimers of scFvs (also known as diabodies) show improved targeting and clearance properties due to their higher molecular weight (55 kDa) and increased avidity. Further gains can be made by generation of larger recombinant fragments, such as the minibody,an scFv-C(H)3 fusion protein that self-assembles into a bivalent dimer of 80 kDa. A systematic evaluation of scFv, diabody, minibody, and intact antibody (based on comparison of tumor uptakes, tumor:blood activity ratios, and calculation of an Imaging Figure of Merit) can form the basis for selection of combinations of recombinant fragments and radionuclides for imaging applications. Ease of engineering and expression, combined with novel specificities that will arise from advances in genomic and combinatorial approaches to target discovery, will usher in a new era of recombinant antibodies for biological imaging.

L28 ANSWER 41 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
2000:618854 The Genuine Article (R) Number: 343GX. **Bispecific** antibodies in cancer therapy. Weiner L M (Reprint). FOX CHASE CANC CTR, DEPT MED ONCOL, 7701 BURHOLME AVE, PHILADELPHIA, PA 19111 (Reprint). CANCER JOURNAL (MAY 2000) Vol. 6, Supp. [3], pp. S265-S271. Publisher: JONES AND BARTLETT PUBLISHERS. 40 TALL PONE DR, SUDBURY, MA 01776. ISSN: 1528-9117. Pub. country: USA. Language: English.

L28 ANSWER 42 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
2000:716283 The Genuine Article (R) Number: 355BD. Effects of unpaired cysteines on yield, solubility and activity of different recombinant antibody constructs expressed in E. coli. Schmiedl A; Breitling F; Winter

C H; Queitsch I; Dubel S (Reprint). UNIV HEIDELBERG, INST GENET MOL, NEUENHEIMER FELD 230, D-69120 HEIDELBERG, GERMANY (Reprint); UNIV HEIDELBERG, INST GENET MOL, D-69120 HEIDELBERG, GERMANY; GERMAN CANC RES CTR, D-69120 HEIDELBERG, GERMANY. JOURNAL OF IMMUNOLOGICAL METHODS (28 AUG 2000) Vol. 242, No. 1-2, pp. 101-114. Publisher: ELSEVIER SCIENCE BV. PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 0022-1759. Pub. country: GERMANY. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB New E. coli vectors based on the pOPE/pSTE vector system [Gene 128 (1993) 97] were constructed to express a **single-chain Fv** antibody fragment (scFv), a scFv-streptavidin fusion protein and two disulfide bond-stabilized Fv antibody fragments (dsFvs) utilizing different side chain positions for disulfide stabilization. All of these constructs encoded fusion proteins carrying five C-terminal histidine residues preceded by an unpaired cysteine. The influence of this cysteine, which was originally introduced to allow the chemical modification of the fusion proteins, was assessed by exchanging the two amino acids CysIle in front of the carboxy terminal His-tag to SerHis in all constructs. Yield and antigen-binding activity of the antibody constructs were compared after standard lab-scale periplasmic expression in Escherichia coli. The removal of the unpaired cysteine resulted in a significant increase in antigen-binding activity of the crude periplasmic extracts. Further, a three-five fold increase or. yield and a significantly improved purity were observed after immobilized metal affinity chromatography (IMAC) with all four constructs.

L28 ANSWER 43 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
1999:409228 The Genuine Article (R) Number: 198VC. Characterization of scFv-Ig constructs generated from the anti-CD20 mAb 1F5 using linker peptides of varying lengths. Shan D M (Reprint); Press O W; Tsu T T; Hayden M S; Ledbetter J A. UNIV WASHINGTON, MED CTR, DIV MED ONCOL, DEPT BIOL STRUCT, BOX 356043, SEATTLE, WA 98195 (Reprint); UNIV WASHINGTON, DEPT MED, SEATTLE, WA 98195; FRED HUTCHINSON CANC RES CTR, SEATTLE, WA 98104; BRISTOL MYERS SQUIBB PHARMACEUT RES INST, SEATTLE, WA 98121. JOURNAL OF IMMUNOLOGY (1 JUN 1999) Vol. 162, No. 11, pp. 6589-6595. Publisher: AMER ASSOC IMMUNOLOGISTS. 9650 ROCKVILLE PIKE, BETHESDA, MD 20814. ISSN: 0022-1767. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The heavy (V-H) and light (V-L) chain variable regions of the murine anti-human CD20 mAb 1F5 were cloned, and four single-chain Ab (scFv) molecules were constructed using linker peptides of variable lengths to join the V-H and V-L domains. Three constructs were engineered using linker peptides of 15, 10, and 5 aa residues consisting of (GGGGS)(3), (GGGGS)(2), and (GGGGS)(1) sequences, respectively, whereas the fourth was prepared by joining the V-H and V-L domains directly. Each construct was fused to a derivative of human IgG1 (hinge plus CH2 plus CH3) to facilitate purification using staphylococcal protein A. The aggregation and CD20 binding properties of these four 1F5 scFv-Ig derivatives produced were investigated. Both size-exclusion HPLC column analysis and Western blots of proteins subjected to nonreducing SDS-PAGE suggested that all four 1F5 scFv-Ig were monomeric with m.w. of similar to 55 kDa, The CD20 binding properties of the four 1F5 scFv-Ig were studied by ELISA and how cytometry, The 1F5 scFv-Ig with the 5-aa linker (GS1) demonstrated significantly superior binding to CD20-expressing target cells, compared with the other scFv-Ig constructs. Scatchard analysis of the radiolabeled monovalent GS1 scFv-Ig revealed a binding avidity of 1.35 x 1(8) M-1 compared with an avidity of 7.56 x 10(8) M-1 for the native bivalent 1F5 Ab, These findings suggest that the GS1 scFv-Ig with a short linker peptide of similar to 5 aa is the best of the engineered constructs for future studies.

L28 ANSWER 44 OF 128 MEDLINE
1999427986 Document Number: 99427986. PubMed ID: 10499640.

Radioimmunotherapy of human colon cancer xenografts using a dimeric **single-chain Fv** antibody construct. Pavlinkova G; Booth B J; Batra S K; Colcher D. (Department of Pathology, University of Nebraska Medical Center, Omaha 68198-3135, USA.. gpavlink@unmc.edu) . CLINICAL CANCER RESEARCH, (1999 Sep) 5 (9) 2613-9. Journal code: 9502500. ISSN: 1078-0432. Pub. country: United States. Language: English.

AB Progress in the use of monoclonal antibodies (MAbs) for the treatment of solid tumors is limited by a number of factors, including poor penetration of the labeled IgG molecule into the tumors, their inability to reach the tumor in sufficient quantities without significant normal tissue toxicity, and the development of a human antimouse antibody response to the injected MAb. One possible way to alter the pharmacology of antibodies is via the use of smaller molecular weight antibody fragments called **single -chain Fvs** (scFvs). A divalent construct of MAb CC49, CC49 (scFv)2, composed of two noncovalently associated scFvs, was generated and shown to bind a tumor-associated antigen (TAG-72) epitope with a similar binding affinity to that of the murine IgG. The therapeutic potential of this construct after labeling with 131I was examined in athymic mice bearing established s.c. human colon carcinoma (LS-174T) xenografts. Treatment groups (n = 10) received a single dose of 131I-labeled CC49 (scFv)2 (500-2000 microCi) or 131I-labeled CC49 IgG (250 and 500 microCi). The group of mice treated with the lowest dose of 131I-(scFv)2 (500 microCi) showed statistically significant prolonged survival, compared with controls (P = 0.036). Complete tumor regression was observed in 20% of mice given 1500 microCi of labeled (scFv)2 and 30 and 60% of mice treated with 250 and 500 microCi of labeled IgG, respectively. In conclusion, the CC49 (scFv)2 construct provides a promising delivery vehicle for therapeutic applications.

L28 ANSWER 45 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)

1999:785731 The Genuine Article (R) Number: 244PM. A **bispecific** diabody that mediates natural killer cell cytotoxicity against xenotransplantated human Hodgkin's tumors. Arndt M A E; Krauss J; Kipriyanov S M; Pfreundschuh M; Little M (Reprint). DEUTSCH KREBSFORSCHUNGSZENTRUM, RECOMBINANT ANTIBODY RES GRP D0500, NEUENHEIMER FELD 280, D-69120 HEIDELBERG, GERMANY (Reprint); DEUTSCH KREBSFORSCHUNGSZENTRUM, RECOMBINANT ANTIBODY RES GRP D0500, D-69120 HEIDELBERG, GERMANY; UNIV CLIN SAARLAND, DEPT INTERNAL MED 1, HOMBURG, GERMANY. BLOOD (15 OCT 1999) Vol. 94, No. 8, pp. 2562-2568. Publisher: W B SAUNDERS CO. INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399. ISSN: 0006-4971. Pub. country: GERMANY. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB CD16/CD30 **bispecific** monoclonal antibodies can induce remissions of Hodgkin's disease refractory to chemo- and radiotherapy. However, the development of human anti-mouse immunoglobulin antibodies and allergic reactions precludes repeated applications of the antibody. Moreover, problems of producing and purifying sufficient amounts of material limit the clinical practicability of this novel treatment approach. To overcome these obstacles, we have constructed a **bispecific** antibody in a diabody form that only employs the variable domains of the CD16/CD30 hybrid hybridoma, The diabody compared favorably with the parent CD16/CD30 **bispecific** antibody in its ability to activate and target natural killer cells in vitro. Its administration to mice bearing xenografted Hodgkin's lymphoma resulted in a marked regression of tumor growth, thus proving for the first time the capability of a diabody for immune recruitment in vivo. The CD16/CD30 diabody is a novel reagent that should considerably facilitate the immunotherapy of patients with refractory Hodgkin's lymphoma. (C) 1999 by The American Society of Hematology.

L28 ANSWER 46 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)

1999:708572 The Genuine Article (R) Number: 234ZU. Pharmacokinetics and

biodistribution of engineered single-chain antibody constructs of MAb CC49 in colon carcinoma xenografts. Pavlinkova G; Beresford G W; Booth B J M; Batra S K; Colcher D (Reprint). COULTER PHARMACEUT INC, 600 GATEWAY BLVD, S SAN FRANCISCO, CA 94080 (Reprint); UNIV NEBRASKA, MED CTR, DEPT PATHOL & MICROBIOL, OMAHA, NE; UNIV NEBRASKA, MED CTR, DEPT BIOCHEM & MOL BIOL, OMAHA, NE. JOURNAL OF NUCLEAR MEDICINE (SEP 1999) Vol. 40, No. 9, pp. 1536-1546. Publisher: SOC NUCLEAR MEDICINE INC. 1850 SAMUEL MORSE DR, RESTON, VA 20190-5316. ISSN: 0161-5505. Pub. country: USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Monoclonal antibodies (MAbs) have been proven useful in clinical studies for both diagnostic and therapeutic applications. The **single-chain Fv** (scFv) construct made from MAbs has potential applications for improved cancer diagnosis and therapy. A new CC49 scFv construct recognizing a tumor-associated mucin, TAG-72, was engineered and evaluated by immunological, pharmacokinetic and biodistribution analysis. Methods: The CC49 scFv construct was generated in which the V-L and V-H variable region genes were joined together with a 25-amino acid helical linker (205C). The new CC49 scFv(205C) was expressed as a monomer as well as a stable noncovalent dimer ([scFv](2)). The pharmacokinetic, biodistribution and tumor targeting characteristics of radiolabeled CC49 scFv were compared with CC49 IgG and enzymatically derived fragments F(ab')(2) and Fab', using the athymic mice bearing human colon cancer xenografts. Results: The association constant (KA) for the intact CC49, dimeric scFv (scFv), and monomeric scFv were 1.7 x 10(9), 1.99 x 10(9) and 0.52 x 10(9) M-1 by Scatchard analysis and 1.14 x 10(8), 4.46 x 10(7) and 1.5 x 10(7) M-1, respectively, by BIAcore analysis. Pharmacokinetic studies showed that more than 50% of monomeric scFv (similar to 27 kDa) was cleared from the blood in less than 10 min. The CC49 Fab' generated enzymatically from the parent murine Mab' (50 kDa) had a blood clearance that was faster than that of the (scFv), (60 kDa) with half of the activity cleared from the serum within 30 and 50 min, respectively. The CC49 dimeric scFv(205C) showed a two-fold higher tumor uptake (than scFv or Fab') reaching 10 %ID/g at 60 min after injection. The scFv dimer also showed an excellent stability and increased avidity in vivo compared with the monomer, as demonstrated by the longer retention in tumor with 3 %ID/g remaining at 48 h. Conclusion: The rapid clearance from the blood, higher tumor uptake and longer retention of the stable dimer of CC49 scFv make it an important agent for potential imaging and therapeutic applications.

L28 ANSWER 47 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)

1999:425298 The Genuine Article (R) Number: 201EC. Binding characteristics and tumor targeting of a covalently linked divalent CC49 single-chain antibody. Beresford G W; Pavlinkova G; Booth B J M; Batra S K; Colcher D (Reprint). UNIV NEBRASKA, MED CTR 983135, DEPT PATHOL & MICROBIOL, OMAHA, NE 68198 (Reprint); UNIV NEBRASKA, MED CTR 983135, DEPT PATHOL & MICROBIOL, OMAHA, NE 68198; UNIV NEBRASKA, MED CTR, DEPT BIOCHEM & MOL BIOL, OMAHA, NE 68198. INTERNATIONAL JOURNAL OF CANCER (11 JUN 1999) Vol. 81, No. 6, pp. 911-917. Publisher: WILEY-LISS. DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012. ISSN: 0020-7136. Pub. country: USA . Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Multivalency is a recognized means of increasing the functional affinity of **single-chain Fvs** (scFvs) for optimizing tumor uptake. A unique divalent **single-chain Fv** protein [sc(Fv)(2)], based on the variable regions of the monoclonal antibody (MAb) CC49, has been generated that differs from other dimeric single-chain constructs in that a linker sequence (L) is encoded between the repeated V-L and V-H domains (V-L-L-V-H-L-V-L-L-V-H). This construct was expressed in soluble form in Escherichia coli and purified by ion-exchange and gel-filtration chromatography, purity and immunoreactivity were determined by SDS-PAGE, HPLC and competitive RIA,

sc(Fv)(2) exhibited a relative K-A (3.34 x 10(7) M-1) similar to that of the native IgG (1.14 x 10(8) M-1) as determined by BIAcore analysis. Pharmacokinetic studies showed rapid blood clearance for sc(Fv)(2), with a T-1/2 less than 40 min. Whole-body clearance analysis also revealed rapid clearance, suggesting no significant retention in the extravascular space or normal tissues. Biodistribution studies of radiolabeled sc(Fv)(2) showed tumor uptake greater than 6% ID/g after 30 min, which remained at this level for 6 hr, High tumor uptake and retention of sc(Fv)(2) coupled with rapid blood and whole-body clearance makes this dimeric scFv of MAb CC49 a strong candidate for imaging and therapeutic applications, (C) 1999 Wiley-Liss, Inc.

L28 ANSWER 48 OF 128 MEDLINE DUPLICATE 14
1999339950 Document Number: 99339950. PubMed ID: 10411643. Expression and characterization of **bispecific single-chain Fv** fragments produced in transgenic plants. Fischer R; Schumann D; Zimmermann S; Drossard J; Sack M; Schillberg S. (Fraunhofer Abteilung fur Molekulare Biotechnologie, IUCT, Grafschaft, Schmallenberg, Germany.. fischer@bio1.rwth-aachen.de) . EUROPEAN JOURNAL OF BIOCHEMISTRY, (1999 Jun) 262 (3) 810-6. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB We describe the expression of the **bispecific** antibody biscFv2429 in transgenic suspension culture cells and tobacco plants. biscFv2429 consists of two single-chain antibodies, scFv24 and scFv29, connected by the Trichoderma reesi cellobiohydrolase I linker. biscFv2429 binds two epitopes of tobacco mosaic virus (TMV): the scFv24 domain recognizes neotopes of intact virions, and the scFv29 domain recognizes a cryptotope of the TMV coat protein monomer. biscFv2429 was functionally expressed either in the cytosol (biscFv2429-cyt) or targeted to the apoplast using a murine leader peptide sequence (biscFv2429-apoplast). A third construct contained the C-terminal KDEL sequence for retention in the ER (biscFv2429-KDEL). Levels of cytoplasmic biscFv2429 expression levels were low. The highest levels of antibody expression were for apoplast-targeted biscFv2429-apoplast and ER-retained biscFv2429-KDEL that reached a maximum expression level of 1.65% total soluble protein in transgenic plants. Plant-expressed biscFv2429 retained both epitope specificities, and bispecificity and bivalency were confirmed by ELISA and surface plasmon resonance analysis. This study establishes plant cells as an expression system for **bispecific** single-chain antibodies for use in medical and biological applications.

L28 ANSWER 49 OF 128 CAPLUS COPYRIGHT 2002 ACS
1999:275550 Document No. 131:57481 Selection of an anti-CD20, single-chain antibody by phage ELISA on fixed cells. Schmidt, Stefanie; Braunagel, Michael; Kurschner, Timo; Little, Melvyn (German Cancer Research Center, Heidelberg, 69120, Germany). BioTechniques, 26(4), 697-700, 702 (English) 1999. CODEN: BTNQDO. ISSN: 0736-6205. Publisher: Eaton Publishing Co..

AB Cloning the correct genes that code for antibody-variable domains from hybridomas is often complicated by the presence of several Ig transcripts, some of them arising from a myeloma cell line. For the rapid functional evaluation of recombinant antibody fragments against cell-surface antigens, the authors established an efficient expression and screening system using phagemid antibodies and fixed cells. VL and VH-polymerase chain reaction (PCR) products, amplified from hybridoma cDNA, were cloned into the phagemid vector pSEX81. After transduction into E. coli and phage rescue, clones were tested for antigen binding using a phage-ELISA procedure with whole cells fixed to ELISA wells. This procedure facilitated the successful cloning of a functional anti-CD20, single-chain antibody from hybridoma cDNA. The CD20 B-lymphocyte surface antigen expressed by B-cell lymphomas is an attractive target for cancer treatment using immunoconjugates or **bispecific** antibodies.

L28 ANSWER 50 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)

1999:755128 The Genuine Article (R) Number: 241BP. Recombinant antibody constructs in cancer therapy. Hudson P J (Reprint). COMMONWEALTH SCI & IND RES ORG, MOL SCI UNIT, COOPERAT RES CTR DIAGNOST TECHNOL, 343 ROYAL PARADE, PARKVILLE, VIC 3052, AUSTRALIA (Reprint). CURRENT OPINION IN IMMUNOLOGY (OCT 1999) Vol. 11, No. 5, pp. 548-557. Publisher: CURRENT BIOLOGY LTD. 34-42 CLEVELAND STREET, LONDON W1P 6LE, ENGLAND. ISSN: 0952-7915. Pub. country: AUSTRALIA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Recombinant antibodies and their fragments now represent over 30% of all biological proteins undergoing clinical trials for diagnosis and therapy. The focus on antibodies as the ideal cancer-targeting reagents recently culminated in approval by the Food and Drugs Administration for the first engineered therapeutic antibodies. In the past year, important advances have been made in the design, selection and production of new types of engineered antibodies. Innovative selection methods have enabled the isolation of high-affinity cancer-targeting and antiviral antibodies, the latter capable of redirecting viruses for gene therapy applications. In other strategies for cancer diagnosis and therapy, recombinant antibody fragments have been fused to radioisotopes, drugs, toxins, enzymes and biosensor surfaces. **Bispecific** antibodies and related fusion proteins have been produced for cancer immunotherapy, effectively enhancing the human immune response in anticancer vaccines and T cell recruitment strategies.

L28 ANSWER 51 OF 128 MEDLINE DUPLICATE 15
1999376336 Document Number: 99376336. PubMed ID: 10449096. Isolation and characterization of an anti-CD16 **single-chain Fv** fragment and construction of an anti-HER2/neu/anti-CD16 **bispecific** scFv that triggers CD16-dependent tumor cytolysis. McCall A M; Adams G P; Amoroso A R; Nielsen U B; Zhang L; Horak E; Simmons H; Schier R; Marks J D; Weiner L M. (Fox Chase Cancer Center, Philadelphia, PA 19111, USA.) MOLECULAR IMMUNOLOGY, (1999 May) 36 (7) 433-45. Journal code: 7905289. ISSN: 0161-5890. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **Bispecific** antibody (bsAb)-based clinical trials of cancer have been conducted primarily using intact murine monoclonal antibody (mAb)-derived molecules. In some of these trials, toxicity resulting from the interactions of antibody Fc domains with cellular Fc receptors has limited the doses of antibody (Ab) that can be employed. Furthermore, human anti-mouse Ab responses prohibit multiple therapy courses. These factors have decreased the efficacy of the bsAb 2B1, which targets the extracellular domains (ECD) of the HER2/neu protooncogene product and the human FcgammaRIII (CD16). To address these obstacles, we have constructed and characterized a fully human gene-fused bsAb from **single-chain Fv** (scFv) molecules specific for HER2/neu and CD16. The human anti-CD16 scFv component, NM3E2, was isolated from a human scFv phage display library. As binding of NM3E2 to human neutrophil-associated CD16 decreased in the presence of plasma IgG, we have concluded that NM3E2 recognizes an epitope in the vicinity of the Fc binding pocket. Furthermore, the NM3E2 scFv was found by surface plasmon resonance-based epitope mapping to share an overlapping epitope with the Leu-11c mAb. The human anti-HER2/neu scFv component, C6.5, which was previously isolated from a human scFv phage display library, was employed as fusion partner for the creation of a **bispecific** scFv (bs-scFv). In the presence of the C6.5 x NM3E2 bs-scFv, peripheral blood lymphocytes promoted significant lysis of human SK-OV-3 ovarian cancer cells overexpressing HER2/neu. Biodistribution studies performed in SK-OV-3 tumor-bearing scid mice revealed that 1% ID/g of 125I-labeled C6.5 x NM3E2 bs-scFv was specifically retained in tumor at 23 h following injection. These results indicated that both scFv components of the bs-scFv retained their function in the fusion protein. This bsAb should overcome some of the problems associated with the 2B1 bsAb. C6.5 x NM3E2 bs-scFv offers promise as a platform for multifunctional binding proteins

with potential clinical applications as a result of its human origin, lack of an Fc domain, ease of production, high level of in vitro tumor cell cytotoxicity and highly selective tumor targeting.

L28 ANSWER 52 OF 128 MEDLINE

1999167366 Document Number: 99167366. PubMed ID: 10066451. SEA-scFv as a bifunctional antibody: construction of a bacterial expression system and its functional analysis. Sakurai N; Kudo T; Suzuki M; Tsumoto K; Takemura S; Kodama H; Ebara S; Teramae A; Katayose Y; Shinoda M; Kurokawa T; Hinoda Y; Imai K; Matsuno S; Kumagai I. (Tohoku University School of Medicine, Tohoku University, Sendai, Japan.) BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1999 Mar 5) 256 (1) 223-30. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB A SEA-antibody **single chain Fv** (SEA-scFv) fusion protein was produced by bacterial expression system in this study. SEA-scFv has both staphylococcal enterotoxin A (SEA) effects and antibody activity directed at the epithelial mucin core protein MUC1, a cancer associated antigen. It was expressed mostly in the cytoplasm as an insoluble form. The gene product was solubilized by guanidine hydrochloride, refolded by conventional dilution method, and purified using metal-chelating chromatography. The resulting SEA-scFv fusion protein preparation was found to react with MUC1 and MHC class II antigens and had the ability to enhance cytotoxicity of lymphokine activated killer cells with a T cell phenotype against a human bile duct carcinoma cell line, TFK-1, expressing MUC1. This genetically engineered SEA-scFv fusion protein promises to be an important reagent for cancer immunotherapy. Copyright 1999 Academic Press.

L28 ANSWER 53 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)

2000:197729 The Genuine Article (R) Number: 290JU. High avidity scFv multimers; diabodies and triabodies. Hudson P J (Reprint); Kortt A A. CSIRO MOL SCI, 343 ROYAL PARADE, PARKVILLE, VIC 3052, AUSTRALIA (Reprint); CRC DIAGNOST TECHNOL, PARKVILLE, VIC 3052, AUSTRALIA. JOURNAL OF IMMUNOLOGICAL METHODS (10 DEC 1999) Vol. 231, No. 1-2, pp. 177-189. Publisher: ELSEVIER SCIENCE BV. PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS . ISSN: 0022-1759. Pub. country: AUSTRALIA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Multivalent recombinant antibody fragments provide high binding avidity and unique specificity to a wide range of target antigens and haptens. This review describes how careful choice of linker length between V-domains creates new types of Fv modules with size, flexibility and valency suited to in vivo imaging and therapy. Further, we review the design of multi-specific Fv modules suited to cross-linking target antigens for cell-recruitment, viral delivery and immunodiagnostics. **Single chain Fv** antibody fragments (scFvs) are predominantly monomeric when the V-H and V-L domains are joined by polypeptide linkers of at least 12 residues. An scFv molecule with a linker of 3 to 12 residues cannot fold into a functional Fv domain and instead associates with a second scFv molecule to form a bivalent dimer (diabody, similar to 60 kDa). Reducing the linker length below three residues can force scFv association into trimers (triabodies, similar to 90 kDa) or tetramers (similar to 120 KDa) depending on linker length, composition and V-domain orientation. The increased binding valency in these scFv multimers results in high avidity (long off-rates). A particular advantage for tumor targeting is that molecules of similar to 60-100 kDa have increased tumor penetration and fast clearance rates compared to the parent Ig. A number of cancer-targeting scFv multimers have recently undergone pre-clinical evaluation for in vivo stability and efficacy. Bi- and tri-specific multimers can be formed by association df different scFv molecules and, in the first examples, have been designed as cross-linking reagents for T-cell recruitment into tumors (immunotherapy) and as red blood cell agglutination reagents (immunodiagnostics).

L28 ANSWER 54 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
1999:962836 The Genuine Article (R) Number: 263WR. Acquired antagonistic activity of a **bispecific** diabody directed against two different epitopes on vascular endothelial growth factor receptor 2. Lu D; Kotanides H; Jimenez X; Zhou Q W; Persaud K; Bohlen P; Witte L; Zhu Z P (Reprint). IMCLONE SYST, DEPT MOL & CELL BIOL, 180 VARICK ST, NEW YORK, NY 10014 (Reprint); IMCLONE SYST, DEPT MOL & CELL BIOL, NEW YORK, NY 10014; IMCLONE SYST, DEPT PROT CHEM, NEW YORK, NY 10014; IMCLONE SYST, DEPT RES, NEW YORK, NY 10014. JOURNAL OF IMMUNOLOGICAL METHODS (19 NOV 1999) Vol. 230, No. 1-2, pp. 159-171. Publisher: ELSEVIER SCIENCE BV. PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 0022-1759. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Bispecific** antibody (BsAb) technology has been successfully used as a means to construct novel antibody (Ab) molecules with increased avidity for binding, by combining two Ab or their fragments directed against different epitopes within the same antigen. Using two single chain antibodies (scFv) isolated from a phage display library, we have constructed a **bispecific** diabody directed against two different epitopes on the extracellular domain (ECD) of human vascular endothelial growth factor receptor 2 (VEGFR2), the kinase-insert domain-containing receptor (KDR). Neither of the parent scFv blocks KDR/VEGF interactions or inhibits VEGF-induced receptor activation. The diabody binds to KDR with an affinity that is 1.5- to 3-fold higher than its parent scFv, mainly due to a much slower dissociation rate (k(off)), which is approximately 17- to 26-fold slower than that of the individual scFv. In addition, the diabody binds simultaneously to, and thus cross-links, the two epitopes on the receptor(s). It is rather unexpected that the diabody effectively blocked KDR/VEGF interactions, and inhibited both VEGF-induced activation of the receptor and mitogenesis of human endothelial cells. Taken together, our results suggest that the diabody is most likely to exert its effect through steric hindrance and/or causing major conformational changes of the receptor. This is the first report on the construction of a **bispecific** diabody with acquired novel antagonistic activity. (C) 1999 Elsevier Science B.V. All rights reserved.

L28 ANSWER 55 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
2000:91705 The Genuine Article (R) Number: 278CZ. Anti-carcinoembryonic antigen (CEA) diabody for rapid tumor targeting and imaging. Wu A M (Reprint); Williams L E; Zieran L; Padma A; Sherman M; Bebb G G; OdomMaryon T; Wong J Y C; Shively J E; Raubitschek A A. CITY HOPE NATL MED CTR, BECKMAN RES INST, DEPT MOL BIOL, 1450 E DUARTE RD, DUARTE, CA 91910 (Reprint); CITY HOPE NATL MED CTR, BECKMAN RES INST, DEPT MOL BIOL, DUARTE, CA 91010; CITY HOPE NATL MED CTR, BECKMAN RES INST, DIV BIOL, DUARTE, CA 91010; CITY HOPE NATL MED CTR, BECKMAN RES INST, DIV IMMUNOL, DUARTE, CA 91010; CITY HOPE NATL MED CTR, DIV RADIOL, DUARTE, CA 91010; CITY HOPE NATL MED CTR, DIV SURG, DUARTE, CA 91010; CITY HOPE NATL MED CTR, DIV RADIAT ONCOL, DUARTE, CA 91010; CITY HOPE NATL MED CTR, DEPT BIOSTAT, DUARTE, CA 91010; CITY HOPE NATL MED CTR, DEPT RADIOIMMUNOTHERAPY, DUARTE, CA 91010. TUMOR TARGETING (APR 1999) Vol. 4, No. 1, pp. 47-58. Publisher: STOCKTON PRESS. HOUNDMILLS, BASINGSTOKE RG21 6XS, HAMPSHIRE, ENGLAND. ISSN: 1351-8488. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Single-chain antibody (scFv) dimers (also known as diabodies) provide a facile route for production of engineered fragments with favorable tumor targeting and biodistribution properties in vivo, A scFv was constructed from the variable regions of the anti-carcinoembryonic antigen (CEA) monoclonal antibody T84.66 using a peptide linker of only eight amino acid residues in order to prevent formation of scFv monomers and produce dimers (diabodies) exclusively, The anti-CEA diabody was secreted at high level from E. coil and was purified by affinity chromatography, The T84.66/GS8

diabody bound antigen bivalently and retained a high apparent affinity for CEA (K-A = 8.19 x 10(10) M-1) as determined by surface plasmon resonance. Targeting properties of I-123-labeled T84.66 diabody were evaluated in vivo using athymic mice bearing LS174T human colorectal carcinoma xenografts. I-123-labeled anti-CPA diabodies exhibited rapid targeting of CPA-positive xenografts, reaching a maximum uptake level of 13.68 +/- 1.49% injected dose per gram 2 h after administration. Rapid blood clearance resulted in high tumor/blood uptake ratios; for example, 9.37 at 6 h and 48.69 at 24 h. Using a gamma camera equipped with a pinhole collimator, LS174T xenografts of masses 0.12 to 0.48 gm were readily imaged 6 h after injection, Performance of the anti-CPA diabody (bivalent, 55 kDa) was compared with previously described cognate fragments of T84.66, namely the scFv (28 kDa, monovalent) and minibody (scFv-C(H)3 dimer; bivalent, 80 kDa) by calculation of imaging figures of merit, Results show that diabodies labeled with short-lived radionuclides such as I-123 Or F-18 Should provide useful rapid clinical imaging agents.

L28 ANSWER 56 OF 128 MEDLINE DUPLICATE 16
1999443899 Document Number: 99443899. PubMed ID: 10512714.
Bispecific tandem diabody for tumor therapy with improved antigen binding and pharmacokinetics. Kipriyanov S M; Moldenhauer G; Schuhmacher J; Cochlovius B; Von der Lieth C W; Matys E R; Little M. (Recombinant Antibody Research Group (D0500), German Cancer Research Center (DKFZ), Heidelberg.. s.kipriyanov@dkfz-heidelberg.de) . JOURNAL OF MOLECULAR BIOLOGY, (1999 Oct 15) 293 (1) 41-56. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: ENGLAND: United Kingdom. Language: English.
AB To increase the valency, stability and therapeutic potential of **bispecific** antibodies, we designed a novel recombinant molecule that is **bispecific** and tetravalent. It was constructed by linking four antibody variable domains (VHand VL) with specificities for human CD3 (T cell antigen) or CD19 (B cell marker) into a single chain construct. After expression in Escherichia coli, intramolecularly folded bivalent **bispecific** antibodies with a mass of 57 kDa (single chain diabodies) and tetravalent **bispecific** dimers with a molecular mass of 114 kDa (tandem diabodies) could be isolated from the soluble periplasmic extracts. The relative amount of tandem diabodies proved to be dependent on the length of the linker in the middle of the chain and bacterial growth conditions. Compared to a previously constructed heterodimeric CD3xCD19 diabody, the tandem diabodies exhibited a higher apparent affinity and slower dissociation from both CD3(+)and CD19(+)cells. They were also more effective than diabodies in inducing T cell proliferation in the presence of tumor cells and in inducing the lysis of CD19(+)cells in the presence of activated human PBL. Incubated in human serum at 37 degrees C, the tandem diabody retained 90 % of its antigen binding activity after 24 hours and 40 % after one week. In vivo experiments indicated a higher stability and longer blood retention of tandem diabodies compared to **single chain Fv** fragments and diabodies, properties that are particularly important for potential clinical applications.

L28 ANSWER 57 OF 128 MEDLINE DUPLICATE 17
1998112835 Document Number: 98112835. PubMed ID: 9446596. Recombinant human **single chain Fv** antibodies recognizing human interleukin-6. Specific targeting of cytokine-secreting cells. Krebs B; Griffin H; Winter G; Rose-John S. (Department of Medicine, Section of Pathophysiology, Johannes Gutenberg-University of Mainz, Obere Zahlbacher Strasse 63, D-55101 Mainz, Germany.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Jan 30) 273 (5) 2858-65. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB A human antibody library was displayed on the surface of filamentous bacteriophage and screened for binding to human interleukin-6 (IL-6). Two antibody-bearing phages were selected that bound IL-6. The

complementary-determining region 3 loops of the variable heavy chains of these two antibodies differed in length and sequence and recognized two distinct epitopes. One of the **single chain Fv** fragments isolated (H1) was found to bind human (but not murine) IL-6 with an affinity comparable to that of the human IL-6 receptor. H1 also recognized newly synthesized human IL-6 intracellularly, as shown by indirect immunofluorescence. H1 did not neutralize human IL-6, and the H1 epitope was mapped to a region of IL-6 not involved in interactions with IL-6, IL-6 receptor, or the signal-transducing protein gp130. To target IL-6-secreting cells, we then constructed a **bispecific** antibody fragment (a diabody) comprising H1 and the antigen binding site of the T-cell activating monoclonal antibody OKT3. The diabody led to T-cell-mediated killing of cells secreting IL-6.

L28 ANSWER 58 OF 128 MEDLINE DUPLICATE 18
1999100998 Document Number: 99100998. PubMed ID: 9885907. Mitogenic properties of a **bispecific single-chain Fv**-Ig fusion generated from CD2-specific mAb to distinct epitopes. Connelly R J; Hayden M S; Scholler J K; Tsu T T; Dupont B; Ledbetter J A; Kanner S B. (Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121, USA.) INTERNATIONAL IMMUNOLOGY, (1998 Dec) 10 (12) 1863-72. Journal code: 8916182. ISSN: 0953-8178. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The combination of anti-CD2 mAb 9.6 and 9-1, specific for distinct epitopes, induces proliferation of resting human T cells. The mitogenic activity of this mAb mixture depends upon accessory cells and the 9-1 mAb Fc domain. To further study the functional properties of these mAb, their variable regions were cloned and expressed as monospecific **single -chain Fv** (scFv) proteins fused to the human IgG1 Fc domain (scFvIg). A novel **bispecific** scFvIg was constructed by cloning the two monospecific scFv binding sites in tandem, with the 9.6 scFv placed N-terminal to the 9-1 scFvIg. Monospecific scFvIg binding to CD2 was comparable to that of the corresponding parental mAb, while the **bispecific** scFvIg exhibited binding activity similar to that of the 9-1 scFvIg. The combination of 9.6 scFvIg and 9-1 mAb was mitogenic, whereas mixtures including the 9-1 scFvIg were non-stimulatory, confirming the unique properties of the 9-1 IgG3 Fc. Without the IgG3 tail, the **bispecific** 9.6/9-1 scFvIg was directly mitogenic and was a more potent mitogen than the mAb mixture, but was accessory cell dependent. Unlike the combination of mAb, the **bispecific** reagent did not directly mobilize calcium in T cells. In comparison to the mAb mixture, **bispecific** 9.6/9-1 scFvIg-mediated stimulation of a mixed lymphocyte reaction was significantly more resistant to inhibition of the CD28 co-stimulatory pathway by the inhibitor CTLA-4-Ig. These results show that expression of the 9.6 and 9-1 binding sites together on a **bispecific** scFvIg increased the mitogenic properties of the mAb and altered the degree of accessory cell signals required for T cell activation.

L28 ANSWER 59 OF 128 MEDLINE DUPLICATE 19
1998349400 Document Number: 98349400. PubMed ID: 9686611. In vivo retargeting of T cell effector function by recombinant **bispecific single chain Fv** (anti-CD3 x anti-idiotype) induces long-term survival in the murine BCL1 lymphoma model. De Jonge J; Heirman C; de Veerman M; Van Meirvenne S; Moser M; Leo O; Thielemans K. (Vrije Universiteit Brussel, Medical School, Laboratory of Physiology-Immunology, Belgium.) JOURNAL OF IMMUNOLOGY, (1998 Aug 1) 161 (3) 1454-61. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB As demonstrated in several preclinical models, **bispecific** Abs are attractive immunotherapeutic agents for tumor treatment. We have previously reported that a bacterially produced anti-CD3 x antitumor **bispecific** single chain variable fragment of Ab fragment (BsscFv),

which is capable of retargeting CTLs toward BCL1 tumor cells, exhibits antitumor activity in vitro. To further facilitate BsscFv production, the coding sequence was subcloned in a eukaryotic expression vector and introduced into Chinese hamster ovary cells for large-scale production. In this report, we have determined the serum stability and the clearance rate from the circulation of BsscFv. Most important, we prove here the therapeutic value of BsscFv in the treatment of BCL1 lymphoma, a murine model for human non-Hodgkin's lymphoma. Tumor-bearing mice that were treated with rscFv in combination with staphylococcal enterotoxin B superantigen, human rIL-2, or murine rIL-12 showed long-term survival, whereas untreated mice all died. This is the first report of the successful in vivo use of BsscFv as an immunotherapeutic agent. Furthermore, long-term survival was the result of complete tumor removal and was not due to the induction of dormancy.

L28 ANSWER 60 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
1998:894462 The Genuine Article (R) Number: 139EX. High-affinity recombinant phage antibodies to the pan-carcinoma marker epithelial glycoprotein-2 for tumour targeting. Roovers R C; Henderikx P; Helfrich W; vanderLinden E; Reurs A; deBruine A P; Arends J W; deLeij L; Hoogenboom H R (Reprint). UNIV HOSP MAASTRICHT, DEPT PATHOL, CESAME, POB 5800, NL-6202 AZ MAASTRICHT, NETHERLANDS (Reprint); UNIV HOSP MAASTRICHT, DEPT PATHOL, CESAME, NL-6202 AZ MAASTRICHT, NETHERLANDS; UNIV GRONINGEN HOSP, DEPT CLIN IMMUNOL, NL-9713 EZ GRONINGEN, NETHERLANDS. BRITISH JOURNAL OF CANCER (DEC 1998) Vol. 78, No. 11, pp. 1407-1416. Publisher: CHURCHILL LIVINGSTONE. JOURNAL PRODUCTION DEPT, ROBERT STEVENSON HOUSE, 1-3 BAXTERS PLACE, LEITH WALK, EDINBURGH EH1 3AF, MIDLOTHIAN, SCOTLAND. ISSN: 0007-0920. Pub. country: NETHERLANDS. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB The tumour-associated antigen epithelial glycoprotein-2 (EGP-2) is a promising target for detection and treatment of a variety of human carcinomas. Antibodies to this antigen have been successfully used in patients for imaging of small-cell lung cancer and for adjuvant treatment of minimal residual disease of colon cancer. We describe here the isolation and complete characterization of high-affinity single-chain variable fragments (scFv) to the EGP-2 antigen. First, the binding kinetics of four murine whole antibodies directed to EGP-2 (17-1A, 323/A3, MOC-31 and MOC-161) were determined using surface plasmon resonance (SPR). The MOC-31 antibody has the lowest apparent off-rate, followed by MOC-161 and 323/A3. The V-genes of the two MOC hybridomas were cloned as scFv in a phage display vector and antigen-binding phage were selected by panning on recombinant antigen. The scFvs compete with the original hybridoma antibodies for binding to antigen and specifically bind to human carcinomas in immunohistochemistry. MOC-31 scFv has an off-rate which is better than those of the bivalent 17-1A and 323/A3 whole antibodies, providing it with an essential characteristic for tumour retention in vivo. The availability of these high-affinity anti-EGP-2 antibody fragments and of their encoding V-genes creates a variety of possibilities for their future use as tumour-targeting vehicles.

L28 ANSWER 61 OF 128 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
1998139160 EMBASE Prolonged in vivo tumour retention of a human diabody targeting the extracellular domain of human HER2/neu. Adams G.P.; Schier R.; McCall A.M.; Crawford R.S.; Wolf E.J.; Weiner L.M.; Marks J.D.. G.P. Adams, Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA 19111, United States. British Journal of Cancer 77/9 (1405-1412) 1998.
Refs: 50.
ISSN: 0007-0920. CODEN: BJCAAI. Pub. Country: United Kingdom. Language: English. Summary Language: English.
AB **Single-chain Fv** (scFv) molecules exhibit highly specific tumour-targeting properties in tumour-bearing mice. However, because of their smaller size and monovalent binding, the

quantities of radiolabelled scFv retained in tumours limit their therapeutic applications. Diabodies are dimeric antibody-based molecules composed of two non-covalently associated scFv that bind to antigen in a divalent manner. In vitro, diabodies produced from the anti-HER2/neu (c-erbB-2) scFv C6.5 displayed approximately 40-fold greater affinity for HER2/neu by surface plasmon resonance biosensor measurements and significantly prolonged association with antigen on the surface of SK-OV-3 cells (t(1/2) cell surface retention of > 5 h vs 5 min) compared with C6.5 scFv. In SK-OV-3 tumour-bearing scid mice, radioiodinated C6.5 diabody displayed a highly favourable balance of quantitative tumour retention and specificity. By as early as 4 h after i.v. administration, significantly more diabody was retained in tumour (10% ID g-1) than in blood (6.7% ID ml-1) or normal tissue (liver, 2.8% ID g-1; lung, 7.1% ID g-1; kidney, 5.2% ID g-1). Over the next 20 h, the quantity present in blood and most tissues dropped approximately tenfold, while the tumour retained 6.5% ID g-1 or about two-thirds of its 4-h value. In contrast, the 24-h tumour retention of radioiodinated C6.5 scFv monomer was only 1% ID g-1. When diabody retentions were examined over the course of a 72-h study and cumulative area under the curve (AUC) values were determined, the resulting tumor-organ AUC ratios were found to be superior to those previously reported for other monovalent or divalent scFv molecules. In conclusion, the diabody format provides the C6.5 molecule with a distinct in vitro and in vivo targeting advantage and has promise as a delivery vehicle for therapeutic agents.

L28 ANSWER 62 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
1999:70125 The Genuine Article (R) Number: 156EA. **Single-chain Fv** with manifold N-glycans as bifunctional scaffolds for immunomolecules. Wang M L; Lee L S; Nepomich A; Yang J D; Conover C; Whitlow M; Filpula D (Reprint). ENZON INC, 20 KINGSBRIDGE RD, PISCATAWAY, NJ 08854 (Reprint); ENZON INC, PISCATAWAY, NJ 08854. PROTEIN ENGINEERING (DEC 1998) Vol. 11, No. 12, pp. 1277-1283. Publisher: OXFORD UNIV PRESS. GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND. ISSN: 0269-2139. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Unlike natural antibodies, **single-chain Fv** (sFv) proteins normally lack asparagine-linked glycosylation. Many designed immunoconjugates and other therapeutics currently employ the advantageous conjugation chemistry or targeting properties provided by the glycoprotein oligosaccharide domain, sFv proteins with engineered N-glycan designs were evaluated in Pichia pastoris for glycosylation efficiency, expression level, oligosaccharide chain length and composition, and affinity, In contrast to nearly all natural glycoproteins? the engineered attachment of N-glycans conveniently near the polypeptide C-terminus was found to produce the optimal results. Furthermore, the percentage modification and chain length of the attached mannose chains were controllable by the use of tandem and overlapping Asn-X-Thr tripeptide sites. The glycosylated sFv mannose chains could be effectively conjugated to polyethylene glycol and the resulting conjugate displayed a 10-fold increased circulating life in mice. The potential to control polymer:sFv or drug:sFv molar ratios by site-specific conjugation may substantially improve the therapeutic efficacy of these minimal antigen-binding molecules.

L28 ANSWER 63 OF 128 MEDLINE DUPLICATE 20
1998351597 Document Number: 98351597. PubMed ID: 9688311.
Bispecific CD3 x CD19 diabody for T cell-mediated lysis of malignant human B cells. Kipriyanov S M; Moldenhauer G; Strauss G; Little M. (Recombinant Antibody Research Group, Diagnostics and Experimental Therapy Program, German Cancer Research Center (DKFZ), Heidelberg.) INTERNATIONAL JOURNAL OF CANCER, (1998 Aug 31) 77 (5) 763-72. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB For the treatment of minimal residual disease in patients with leukemias and malignant lymphomas, we constructed a heterodimeric diabody specific for human CD19 on B cells and CD3epsilon chain of the T cell receptor complex. The **bispecific** diabody was expressed in Escherichia coli using a vector containing a dicistronic operon for co-secretion of V(H)3-V(L)19 and V(H)19-V(L)3 **single-chain Fv** fragments (scFv). It was purified in one step by immobilized metal affinity chromatography (IMAC) from the periplasmic extract and culture medium. Flow cytometry experiments revealed specific interactions of the diabody with both CD3 and CD19 positive cells, to which it bound with affinities close to those of the parental scFvs. It was less stable than anti-CD3 scFv but more stable than anti-CD19 scFv when incubated in human serum at 37 degrees C. In cytotoxicity tests, the diabody proved to be a potent agent for retargeting peripheral blood lymphocytes to lyse tumor cells expressing the CD19 antigen. The efficiency of cell lysis compared favorably with that obtained with a **bispecific** antibody (BsAb) of the same dual specificity that was prepared by the quadroma technique.

L28 ANSWER 64 OF 128 MEDLINE DUPLICATE 21
1998272268 Document Number: 98272268. PubMed ID: 9610737. Targeting T cells against brain tumors with a **bispecific** ligand-antibody conjugate. Roy E J; Cho B K; Rund L A; Patrick T A; Kranz D M. (Department of Biochemistry, University of Illinois, Urbana 61801-3792, USA.. e-roy@uiuc.edu) . INTERNATIONAL JOURNAL OF CANCER, (1998 May 29) 76 (5) 761-6. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB High-affinity receptors expressed on the surface of some tumors can be exploited by chemically conjugating the ligand for the receptor and an antibody against immune effector cells, thus redirecting their cytolytic potential against the tumor. Ovarian carcinomas and some brain tumors express the high-affinity folate receptor (FR). In this report, a transgenic mouse model that generates endogenously arising choroid plexus tumors was used to show that folate/anti-T-cell receptor antibody conjugates can direct infiltration of T cells into solid brain tumor masses. An engineered **single-chain Fv** form of the anti-T-cell receptor antibody KJ16 was conjugated with folate, to produce a **bispecific** agent that was substantially smaller than most previously characterized **bispecific** antibodies. Folate conjugation to the antibody increased T-cell infiltration into the tumors by 10- to 20-fold, and significantly prolonged survival of the mice.

L28 ANSWER 65 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
1998:779076 The Genuine Article (R) Number: 125BM. Neurosurgery and molecular biology: (series 7) molecular biology of antibodies. Yoshikawa K (Reprint); Yoshida J; Nakayashiki N. AICHI MED UNIV, DEPT PATHOL 2, NAGAKUTE, AICHI 4801195, JAPAN; NAGOYA UNIV, SCH MED, DEPT NEUROSURG, NAGOYA, AICHI, JAPAN. NEUROLOGICAL SURGERY (SEP 1998) Vol. 26, No. 9, pp. 758-767. Publisher: IGAKU-SHOIN LTD. 5-24-3 HONGO BUNKYO-KU, TOKYO 113 91, JAPAN. ISSN: 0301-2603. Pub. country: JAPAN. Language: Japanese.

L28 ANSWER 66 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
1998:855448 The Genuine Article (R) Number: 135LP. Surface plasmon resonance biosensors as a tool in antibody engineering. Alfthan K (Reprint). VTT BIOTECHNOL & FOOD RES, POB 1500, FIN-02044 ESPOO, VTT, FINLAND (Reprint). BIOSENSORS & BIOELECTRONICS (15 SEP 1998) Vol. 13, No. 6, pp. 653-663. Publisher: ELSEVIER ADVANCED TECHNOLOGY. OXFORD FULFILLMENT CENTRE THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, OXON, ENGLAND. ISSN: 0956-5663. Pub. country: FINLAND. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Modern gene technology combined with efficient microbial expression systems provides tools to produce antibodies with reduced functional size and improved binding properties as well as antibody fusions or novel antibodies. Surface plasmon resonance based biosensors, which measure

antigen-antibody interactions in real-time, can be used for a diverse characterization of the modified antibodies. To date, the majority of published work originates from real-time biospecific interaction analysis based on the BIAcore(R) instruments. This article describes the range of applications in antibody engineering in which BIAcore has been applied.

L28 ANSWER 67 OF 128 MEDLINE DUPLICATE 22
1999034503 Document Number: 99034503. PubMed ID: 9815155.
Bispecific antibodies as novel bioconjugates. Cao Y; Suresh M R. (Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada, T6G 2N8.) BIOCONJUGATE CHEMISTRY, (1998 Nov-Dec) 9 (6) 635-44. Ref: 119. Journal code: 9010319. ISSN: 1043-1802. Pub. country: United States. Language: English.

AB **Bispecific** antibodies are unique macromolecular heterobifunctional cross-linkers with two different binding specificities within a single molecule. As ideal bioconjugates, they can specifically glue any two different molecules together without the need for chemical conjugation. With this unique feature, they have immense potential in biological and immunological fields. Their applications range from immunohistochemistry, immunoassays, radioimmunodiagnosis, radioimmunotherapy, and immunotherapy. Recently, a new second generation of **bispecific** molecules, **bispecific single chain Fv** and diabodies, has been produced by DNA recombinant technology. They can be considered as the ultimate magic bullets for in vivo applications. They may theoretically improve tumor or pathogen targeting and minimize side effects, eventually replacing the full-length **bispecific** antibodies. Emphasizing on developmental methodology and clinical applications of **bispecific** antibodies, this review gives a bird's-eye view of these unique bioconjugates.

L28 ANSWER 68 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
1998:650873 The Genuine Article (R) Number: 112UR. A bivalent disulfide-stabilized Fv with improved antigen binding to erbB2. Bera T K; Onda M; Brinkmann U; Pastan I (Reprint). NCI, MOL BIOL LAB, NIH, BLDG 37, ROOM 4E16, 37 CONVENT DR MSC 4255, BETHESDA, MD 20892 (Reprint); NCI, MOL BIOL LAB, NIH, BETHESDA, MD 20892. JOURNAL OF MOLECULAR BIOLOGY (21 AUG 1998) Vol. 281, No. 3, pp. 475-483. Publisher: ACADEMIC PRESS LTD. 24-28 OVAL RD, LONDON NW1 7DX, ENGLAND. ISSN: 0022-2836. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We have used protein engineering to generate a stable bivalent Fv molecule of the anti-erbB2 monoclonal antibody e23. The V-H and V-L domains of the Fv are Linked to each other by a disulfide bond and the two Fvs are connected by a flexible 15 amino acid residue (Gly(4)-Ser)(3) linker. The e23 (dsFv)(2) molecule is fused to a truncated form of Pseudomonas exotoxin to generate a bivalent disulfide-stabilized, (dsFv)(2), immunotoxin. The immunotoxin was expressed in Escherichia coil, refolded in vitro and purified to about 95% purity. Binding studies demonstrated that the (dsFv)(2) molecule has a much higher affinity for erbB2 than a monovalent dsFv molecule and a similar binding affinity as the parental antibody e23. The (dsFv)(2) immunotoxin was 5 to 20-fold more cytotoxic to two e23 antigen-positive cell Lines than the monovalent dsFv immunotoxin. The bivalent dsFv molecule is very stable, retaining 94% of its activity after a 24 hours incubation in human serum at 37 degrees C. Two other molecules with shorter linkers five and ten amino acid residues in length were produced and showed similar activities as the molecule containing a 15 amino acid residue linker. The bivalency, stability and the relative ease of purification makes these e23 (dsFv)(2) molecules valuable reagents for cancer immunotherapy and diagnosis.

L28 ANSWER 69 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
1998:470216 The Genuine Article (R) Number: ZT936. Biological therapy of

ovarian cancer: Current directions. Bookman M A (Reprint). FOX CHASE CANC CTR, DEPT MED ONCOL, 7701 BURHOLME AVE, PHILADELPHIA, PA 19111 (Reprint). SEMINARS IN ONCOLOGY (JUN 1998) Vol. 25, No. 3, pp. 381-396. Publisher: W B SAUNDERS CO. INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399. ISSN: 0093-7754. Pub. country: USA. Language: English.

L28 ANSWER 70 OF 128 MEDLINE DUPLICATE 23
1998149664 Document Number: 98149664. PubMed ID: 9490020. The first constant domain (C(H)1 and C(L)) of an antibody used as heterodimerization domain for **bispecific** miniantibodies. Muller K M; Arndt K M; Strittmatter W; Pluckthun A. (Biochemisches Institut, Universitat Zurich, Switzerland.) FEBS LETTERS, (1998 Jan 30) 422 (2) 259-64. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.
AB **Bispecific** miniantibodies were constructed by genetically fusing the C(H)1 domain of an IgG1 to the C-terminus of a **single-chain Fv** fragment (scFv-425), specific for the EGF receptor, and fusing the C(L) domain of a kappa light chain to the C-terminus of a scFv specific for CD2 (scFv-M1). An efficient dicistronic gene arrangement for functional expression in Escherichia coli was constructed. Immunoblots demonstrated correct domain assembly and the formation of the natural C(H)1-C(L) disulfide bridge. Gel filtration confirmed the correct size, sandwich ELISAs demonstrated **bispecific** functionality, and SPR biosensor measurements determined binding to EGF-R in comparison to bivalent constructs. **Bispecific** anti-EGF-R/anti-CD2 miniantibodies are candidates for the immunotherapy of cancer.

L28 ANSWER 71 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
1999:59550 The Genuine Article (R) Number: 154PA. Pharmacokinetics and biodistribution of genetically-engineered antibodies. Colcher D (Reprint); Pavlinkova G; Beresford G; Booth B J M; Choudhury A; Batra S K. UNIV NEBRASKA, MED CTR, DEPT PATHOL & MICROBIOL, 600 S 42ND ST, OMAHA, NE 68198 (Reprint); UNIV NEBRASKA, MED CTR, DEPT BIOCHEM & MOL BIOL, OMAHA, NE 68198. QUARTERLY JOURNAL OF NUCLEAR MEDICINE (DEC 1998) Vol. 42, No. 4, pp. 225-241. Publisher: EDIZIONI MINERVA MEDICA. CORSO BRAMANTE 83-85 INT JOURNALS DEPT., 10126 TURIN, ITALY. ISSN: 1125-0135. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Monoclonal antibodies (MAbs), because of their inherent specificity, are ideal targeting agents. They can be used to deliver radionuclides, toxins or cytotoxic drugs to a specific tissue or malignant cell populations. Intact immunoglobulin (IgG) molecules have several practical limitations of their pharmacology; their relatively large size of approximately 150,000 daltons leads to a slow clearance from the blood pool and the body resulting in significant exposure to normal organs with limited quantities delivered to tumors, The IgG molecule shows a relatively poor diffusion from the vasculature into and through the tumor. Attempts to modify the pharmacology of the Ig molecule have classically involved the use of proteases to generate F(ab')(2) and Fab' fragments with molecular weights of approximate to 100,000 and 50,000 daltons, respectively. Fv fragments of IgG are one of the smallest size functional modules of antibodies that retain high affinity binding of an antigen. Their smaller size, approximate to 25,000 daltons, enables better tumor penetration and makes them potentially more useful than a whole antibody molecule for clinical applications. Molecular cloning and expression of the variable region genes of IgG has greatly facilitated the generation of engineered antibodies. A **single-chain Fv** (scFv) recombinant protein, prepared by connecting genes encoding for heavy-chain and light-chain variable regions at the DNA level by an appropriate oligonucleotide linker, clears from the blood at much faster rate than intact IgG, The scFv fragment can retain an antigenbinding affinity similar to that of a monovalent Fab' fragment; this however,

represents a relative decrease in binding affinity when compared to intact antibodies. The scFv with its faster clearance and lower affinity results in a lower percent-injected dose localizing in tumors when compared to the divalent IgG molecule. This may be adequate for imaging but probably not for therapy. The valency of the MAb fragment is critical for the functional affinity of an antibody to a cell surface or a polymeric antigen, In attempts to generate multivalent forms of scFv molecules, noncovalently linked scFv dimeric and trimeric molecules, disulfide linked dimeric scFvs, as well as covalently linked chimeric scFvs have been studied, These multivalent scFvs generally have a higher functional affinity than the monovalent form resulting in better in vivo targeting. Another way to alter the pharmacology of the scFvs is to modify its net charge. Charge-modified scFvs with desired isoelectric points (pI), have been prepared by inserting negatively charged amino acids on the template of the variable region genes. This can help to overcome undesirable elevations in renal uptake seen with most antibody fragments, In conclusion, genetic manipulations of the immunoglobulin molecules are effective means of altering stability, functional affinity, pharmacokinetics, and biodistribution of the antibodies required for the generation of the ''magic bullet''.

L28 ANSWER 72 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)

1998:355323 The Genuine Article (R) Number: ZL317. **Bispecific** antibodies for treatment of cancer in experimental animal models and man. Kroesen B J; Helfrich W; Molema G; deLeij L (Reprint). UNIV GRONINGEN HOSP, DEPT CLIN IMMUNOL, HANZEPLEIN 1, NL-9713 GZ GRONINGEN, NETHERLANDS (Reprint); UNIV GRONINGEN HOSP, DEPT CLIN IMMUNOL, NL-9713 GZ GRONINGEN, NETHERLANDS. ADVANCED DRUG DELIVERY REVIEWS (6 APR 1998) Vol. 31, No. 1-2, pp. 105-129. Publisher: ELSEVIER SCIENCE BV. PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 0169-409X. Pub. country: NETHERLANDS. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Immunotherapy is a powerful anti-cancer treatment modality. However, despite numerous encouraging results obtained in pre-clinical studies, a definite breakthrough towards an established clinical treatment modality has as yet not occurred. Antibodies against tumor antigens have been shown to localise at the site of the tumor, but inadequate triggering of immune effector mechanisms have thwarted clinical efficacy thus far. Cellular immunotherapy has been hampered by limitations such as lack of specificity, down-regulation of major histocompatibility complex (MHC)-expression or Fas ligand up-regulation on tumor cells. This review focuses on the use of **bispecific** antibodies (BsAbs) for immunotherapy of cancer. Using BsAbs, it is possible to take advantage of the highly specific binding characteristics of antibodies and combine these with the powerful effector functions of cytotoxic immune effector cells. BsAbs share two different, monoclonal antibody-derived, antigen-recognizing moieties within one molecule. By dual binding, BsAbs reactive with a trigger molecule on an immune effector cell on the one hand and a surface antigen on a tumor target cell on the other are thus able to functionally focus the lytic activity of the immune effector cell towards the target cell. Over the last few years, the concept of BsAb-mediated tumor cell killing has been studied extensively both in preclinical models and in a number of phase I clinical trials. Promising pre-clinical results have been reported using tumor models in which diverse immune effector cell populations have been used. Despite this pre-clinical in vivo efficacy, the first clinical trials indicate that we are still not in a position to successfully treat human malignancies. This review discusses the production of BsAbs, the choice of trigger molecules in combination with potential effector cells and the preclinical models that have led to the current use of BsAbs in experimental clinical trials. It has become clear that appropriate immune cell activation and establishing a favourable effector-to-target cell ratio will have direct impact on the efficacy of the therapeutic approaches using BsAbs. New

directions are discussed, i.e. finding appropriate dosage schemes by which immune effector cells become redirected without inducing hyporesponsiveness, defining possibilities for combining different immune effector cell populations and creating an in situ tumor environment that allows maximal tumoricidal activity. (C) 1998 Elsevier Science B.V.

L28 ANSWER 73 OF 128 MEDLINE DUPLICATE 24
1998412946 Document Number: 98412946. PubMed ID: 9741915. Targeting tumor cells with **bispecific** antibodies and T cells. Kranz D M; Manning T C; Rund L A; Cho B K; Gruber M M; Roy E J. (Department of Biochemistry, University of Illinois, Urbana 61801, USA.. d-kranz@uiuc.edu) . JOURNAL OF CONTROLLED RELEASE, (1998 Apr 30) 53 (1-3) 77-84. Journal code: 8607908. ISSN: 0168-3659. Pub. country: Netherlands. Language: English.

AB It has been known for some time that mammalian immune systems are capable of eliminating large tumor burdens. Redirecting the immune response of a patient to an established tumor has now become the focus of various therapeutic strategies. In this report, two projects toward this goal are described. The first project involves the development of a transgenic mouse model for T cell directed therapeutics. These mice express specific T cell receptor alpha and beta transgenes on a background in which the recombinational-activating-gene-1 (RAG) has been knocked out. The mice express cytotoxic T cells but not either T helper cells or B cells. Despite these deficiencies, the animals are capable of eliminating tumors that express the appropriate peptide/major histocompatibility complex ligand that is recognized by the alphabeta transgenic T cell receptor. Human tumors grow as transplants in these mice, thereby allowing various agents that redirect the endogenous T cells against human tumors to be tested. The second project involves a description of such agents: **bispecific** antibodies that simultaneously bind to an immune effector cell and a tumor cell. The **bispecific** antibody described here consists of folate attached to anti-T cell receptor antibodies, or their fragments. A **single-chain Fv** coupled with folate can redirect the lysis of human tumor cells that bear the high affinity folate receptor. Preliminary in vivo data showed that the folate/antibody conjugates were also capable of mediating rejection of the human tumor. This transgenic mouse model should now allow the evaluation and optimization of **bispecific** agents that can redirect a patient's own T cell response.

L28 ANSWER 74 OF 128 MEDLINE DUPLICATE 25
1998374022 Document Number: 98374022. PubMed ID: 9710248. A dimeric **bispecific** miniantibody combines two specificities with avidity. Muller K M; Arndt K M; Pluckthun A. (Biochemisches Institut, Universitat Zurich, Switzerland.) FEBS LETTERS, (1998 Jul 31) 432 (1-2) 45-9. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB **Bispecific** antibodies extend the capabilities of nature and might be applied in immunotherapy and biotechnology. By fusing the gene of a **single-chain Fv** (scFv) fragment to a helical dimerization domain, followed by a second scFv fragment of different specificity, we were able to express a functional protein in E. coli, which is **bispecific** and has two valencies for each specificity. The dimeric **bispecific** (DiBi) miniantibody preserves the natural avidity of antibodies in a very small-sized molecule of only 120 kDa. The generality of the principle was shown with a scFv fragment binding the EGF-receptor (named scFv 425) in three combinations with scFv fragments either directed against CD2 (ACID2.M1), phosphorylcholine (McPC603) or fluorescein (FITC-E2). Binding was analyzed by sandwich surface plasmon resonance biosensor (BIAcore) measurements.

L28 ANSWER 75 OF 128 MEDLINE DUPLICATE 26
1998022791 Document Number: 98022791. PubMed ID: 9354666. A recombinant human scFv anti-Rh(D) antibody with multiple valences using a C-terminal

fragment of C4-binding protein. Libyh M T; Goossens D; Oudin S; Gupta N; Dervillez X; Juszczak G; Cornillet P; Bougy F; Reveil B; Philbert F; Tabary T; Klatzmann D; Rouger P; Cohen J H. (Laboratoire d'Immunologie, UFR Medecine, Pole Biomolecules URCA, Reims, France.) BLOOD, (1997 Nov 15) 90 (10) 3978-83. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Monomeric recombinant molecules prove generally unsatisfactory for in vivo use. Most biological systems are indeed multivalent either structurally, associating different chains, or functionally, when cross-linked by their ligands. Mimicking natural molecules for immune intervention implies the need for multimerizing systems to create multivalent molecules capable of interfering with physiological processing. A multivalent anti-Rh(D) recombinant protein has been designed by reconstructing the antibody binding site of a human monoclonal anti-Rh(D) antibody as a **single chain Fv** mini antibody, then multimerizing it by inserting at its C-terminal end the C-terminal part of the C4 binding protein (C4bp) alpha chain, which is responsible for the octamer multimerization of that molecule. This soluble multivalent recombinant molecule was functional, bound red blood cells (RBCs), agglutinated them, and did not activate complement. This demonstration model opens the way for future in vivo use of multivalent molecules associating antibody valences and other functional molecules for cell targeting, imaging, or removal of cells such as Rh(D)-positive RBCs for preventing Rh alloimmunization.

L28 ANSWER 76 OF 128 CAPLUS COPYRIGHT 2002 ACS

1997:347872 Document No. 127:79959 Design and expression of a stable **bispecific** scFv dimer with affinity for both glycophorin and N9 neuraminidase. Atwell, John L.; Pearce, Lesley A.; Lah, Maria; Gruen, L. Clem; Kortt, Alexander A.; Hudson, Peter J. (Division of Biomol. Engineering and CRC Diagnostic Technologies, CSIRO, Parkville, 3052, Australia). Molecular Immunology, Volume Date 1996, 33(17/18), 1301-1312 (English) 1997. CODEN: MOIMD5. ISSN: 0161-5890. Publisher: Elsevier.

AB The authors have designed and produced a stable **bispecific** scFv dimer (bisFv) by non-covalent assocn. of two hybrid VH-VL pairs derived from an anti-neuraminidase antibody (NC10) and an anti-glycophorin antibody (1C3). The bisFv dimer was demonstrated to have binding activity to the two resp. target antigens and was evaluated as a reagent for rapid whole blood agglutination assays. The bisFv was expressed in the periplasm of Escherichia coli, from a secretion vector which comprised two cistrons in tandem under the control of a single lac promoter, inducible with IPTG. Each cistron encoded one of the hybrid VH-VL pairs, with V domains sepd. by a linker region encoding the 5 amino acids, Gly4Ser. The short linker region was designed to prevent assocn. of VH and VL regions of the same mol. and favor the formation of dimers. The protein synthesized from each hybrid scFv cistron was directed to the E. coli periplasm by the inclusion of distinctive signal secretion sequences preceding each hybrid gene; from pel B of Erwinia cartovora and from gene III of fd phage. The bisFv was affinity-purified from culture supernatants via the C-terminal tag epitope FLAG and was shown, by FPLC on a Superose 6 column, to be consistent in size with that of a scFv dimer. The bisFv was stable for >4 mo at 4.degree. and was shown by BIAcore anal. to bind to either target antigen, human glycophorin, or tern N9 neuraminidase. Simultaneous binding to both target antigens was demonstrated when a pre-formed bis Fv-neuraminidase complex was shown to bind to immobilized glycophorin. In whole blood agglutination assays, the bisFv dimer was able to agglutinate red blood cells when crosslinked with anti-idiotype antibody (3-2G12) binding to the NC10 combining site, but no agglutination occurred on binding the antigen neuraminidase. These results are a function of the topol. of the epitopes on neuraminidase and have implications for the use of relatively rigid bifunctional mols. (as bisFv dimers) to cross-link two large membrane-anchored moieties, in this case, red blood cell glycophorin and neuraminidase, an Mr 190,000

tetramer.

L28 ANSWER 77 OF 128 CAPLUS COPYRIGHT 2002 ACS
1997:718994 Document No. 128:21612 Linear gene fusions of antibody fragments with streptavidin can be linked to biotin labeled secondary molecules to form **bispecific** reagents. Pearce, Lesley A.; Oddie, Geoffrey W.; Coia, Gregory; Kortt, Alexander A.; Hudson, Peter J.; Lilley, Glenn G. (Division of Biomolecular Engineering, CSIRO, Parkville, 3052, Australia). Biochemistry and Molecular Biology International, 42(6), 1179-1188 (English) 1997. CODEN: BMBIES. ISSN: 1039-9712. Publisher: Academic.
AB Monomeric single chain antibody (scFv) fragments lack both the avidity of the bivalent IgG, or (Fab')2 fragment, and the effector functions conferred by the Fc domain. For certain diagnostic or therapeutic applications it may be desirable to link these mols. to other proteins, antibodies, enzymes or peptide ligands, and chem. or recombinant methods have been developed to produce many of these crosslinked reagents. One approach has been to link an antibody fragment to streptavidin which can bind a second biotinylated mol. to create a higher affinity, bifunctional or **bispecific** mol. To demonstrate the applicability of this technol., an anti-neuraminidase NC10 scFv-streptavidin fusion was expressed in E. coli and the product was refolded and purified to homogeneity from 6 M guanidine hydrochloride. Anal. in a BIAcoreTM biosensor showed that the NC10 scFv moiety reacted with immobilized neuraminidase and that the core streptavidin moiety was able to bind biotinylated anti-ferritin Fab' to produce a new model **bispecific** reagent which bound ferritin. Conceptually, this design principle can be applied to the creation of useful diagnostic and possibly therapeutic mols.

L28 ANSWER 78 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1997:419161 Document No.: PREV199799718364. Design and evaluation of **bispecific** miniantibodies. Muller, K. M.; Arndt, K. M.; Pluckthun, A.. Dep. Biochem., Univ. Zurich, 8057 Zurich Switzerland. FASEB Journal, (1997) Vol. 11, No. 9, pp. A836. Meeting Info.: 17th International Congress of Biochemistry and Molecular Biology in conjunction with the Annual Meeting of the American Society for Biochemistry and Molecular Biology San Francisco, California, USA August 24-29, 1997 ISSN: 0892-6638. Language: English.

L28 ANSWER 79 OF 128 MEDLINE
97335926 Document Number: 97335926. PubMed ID: 9192721. An engineered bivalent single-chain antibody fragment that increases antigen binding activity. Luo D; Geng M; Noujaim A A; Madiyalakan R. (Biotechnology Research and Development, AltaRex Inc., University of Alberta, Edmonton, Canada.) JOURNAL OF BIOCHEMISTRY, (1997 May) 121 (5) 831-4. Journal code: 0376600. ISSN: 0021-924X. Pub. country: Japan. Language: English.
AB Bivalent **single chain Fv** (scFv) was constructed by fusing a polypeptide extension containing one or two cysteines to the COOH-terminus of an scFv antibody fragment. The scFv protein was expressed and secreted in a recombinant Pichia pastoris system as a dimer with a C-terminal disulfide bridge, as determined by Western blot analysis under non-reducing conditions. We found that the scFv construct with one cysteine in the C-extension (scFv-1Cys) exhibited a much higher dimer/monomer ratio than the two cysteine counterpart (scFv-2Cys). Binding activity measurements performed by means of a competitive radioimmunoassay showed that scFv-1Cys exhibited specific antigen binding activity, which was almost the same as that of the parental MAb, and approximately four- and fortyfold higher than those of the control scFv monomer and scFv-2Cys.

L28 ANSWER 80 OF 128 MEDLINE DUPLICATE 27
97253444 Document Number: 97253444. PubMed ID: 9098887. Remodeling domain interfaces to enhance heterodimer formation. Zhu Z; Presta L G; Zapata G;

Carter P. (Department of Molecular Oncology, Genentech Inc., South San Francisco, California 94080, USA.) PROTEIN SCIENCE, (1997 Apr) 6 (4) 781-8. Journal code: 9211750. ISSN: 0961-8368. Pub. country: United States. Language: English.

AB An anti-p185HER2/anti-CD3 humanized **bispecific** diabody was previously constructed from two cross-over **single-chain Fv** in which YH and VL domains of the parent antibodies are present on different polypeptides. Here this diabody is used to evaluate domain interface engineering strategies for enhancing the formation of functional heterodimers over inactive homodimers. A disulfide-stabilized diabody was obtained by introducing two cysteine mutations, VL L46C and VH D101C, at the anti-p185HER2.VL/VH interface. The fraction of recovered diabody that was functional following expression in Escherichia coli was improved for the disulfide-stabilized compared to the parent diabody (> 96% versus 72%), whereas the overall yield was > 60-fold lower. Eleven "knob-into-hole" diabodies were designed by molecular modeling of sterically complementary mutations at the two VL/VH interfaces. Replacements at either interface are sufficient to improve the fraction of functional heterodimer, while maintaining overall recoverable yields and affinity for both antigens close to that of the parent diabody. For example, diabody variant v5 containing the mutations VL Y87A:F98M and VH V37F:L45W at the anti-p185HER2 VL/VH interface was recovered as 92% functional heterodimer while maintaining overall recovered yield within twofold of the parent diabody. The binding affinity of v5 for p185HER2 extracellular domain and T cells is eightfold weaker and twofold stronger than for the parent diabody, respectively. Domain interface remodeling based upon either sterically complementary mutations or interchain disulfide bonds can facilitate the production of a functional diabody heterodimer. This study expands the scope of domain interface engineering by demonstrating the enhanced assembly of proteins interacting via two domain interfaces.

L28 ANSWER 81 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)

97:618063 The Genuine Article (R) Number: XQ922. Mammalian cell expression of dimeric small immune proteins (SIP). Li E Q; Pedraza A; Bestagno M; Mancardi S; Sanchez R; Burrone O (Reprint). INT CTR GENET ENGN & BIOTECHNOL, AREA SCI PK, PADRICIANO 99, I-34012 TRIESTE, ITALY (Reprint); INT CTR GENET ENGN & BIOTECHNOL, I-34012 TRIESTE, ITALY. PROTEIN ENGINEERING (JUN 1997) Vol. 10, No. 6, pp. 731-736. Publisher: OXFORD UNIV PRESS. GREAT CLARENDON ST, OXFORD, ENGLAND OX2 6DP. ISSN: 0269-2139. Pub. country: ITALY. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We have designed and expressed bivalent small immune proteins (SIP) based on scFv fragments connected through a short linker of four amino acids to the CH3 domain of the human immunoglobulin gamma 1 H-chain. Three different versions have been designed and expressed in mammalian cells. In one construct a cysteine residue was included in the last amino acid of the flexible 15-amino acid long linker connecting the V-L and V-H domains, thus creating a disulphide bond stabilized molecule. A version with a shorter (five amino acids) V-L/V-H linker was also produced and shown to be efficiently assembled and secreted. All three SIPs form dimers retaining their antigenic specificity in Western blotting and having a comparable functional affinity (avidity) as determined by ELISA.

L28 ANSWER 82 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)

97:456830 The Genuine Article (R) Number: XD510. Two amino acid mutations in an anti-human CD3 **single chain Fv** antibody fragment that affect the yield on bacterial secretion but not the affinity . Kipriyanov S M; Moldenhauer G; Martin A C R; Kupriyanova O A; Little M (Reprint). GERMAN CANC RES CTR DKFZ, RECOMBINANT ANTIBODY RES GRP 0445, DIAGNOST & EXPT THERAPY PROGRAMME, D-69120 HEIDELBERG, GERMANY (Reprint); GERMAN CANC RES CTR DKFZ, RECOMBINANT ANTIBODY RES GRP 0445, DIAGNOST & EXPT THERAPY PROGRAMME, D-69120 HEIDELBERG, GERMANY; GERMAN CANC RES CTR

DKFZ, TUMOR IMMUNOL PROGRAMME, DEPT MOL IMMUNOL 0740, D-69120 HEIDELBERG, GERMANY; UNIV LONDON UNIV COLL, DEPT BIOCHEM & MOL BIOL, BIOMOL STRUCT & MODELING UNIT, LONDON WC1E 6BT, ENGLAND. PROTEIN ENGINEERING (APR 1997) Vol. 10, No. 4, pp. 445-453. Publisher: OXFORD UNIV PRESS. GREAT CLARENDON ST, OXFORD, ENGLAND OX2 6DP. ISSN: 0269-2139. Pub. country: GERMANY; ENGLAND. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Recombinant antibody fragments directed against cell surface antigens have facilitated the development of novel therapeutic agents. As a first step in the creation of cytotoxic immunoconjugates, we constructed a **single-chain Fv** fragment derived from the murine hybridoma OKT3, that recognizes an epitope on the epsilon-subunit of the human CD3 complex. Two amino acid residues were identified that are critical for the high level production of this scFv in Escherichia coil, First, the substitution of glutamic acid encoded by a PCR primer at position 6 of V-H framework 1 by glutamine led to a more than a 30-fold increase in the production of soluble scFv, Second, the substitution of cysteine by a serine in the middle of CDR-H3 additionally doubled the yield of soluble antibody fragment without any adverse effect on its affinity for the CD3 antigen, The double mutant scFv (Q,S) proved to be very stable in vitro: no loss of activity was observed after storage for 1 month at 4 degrees C, while the activity of scFv containing a cysteine residue in CDR-H3 decreased by more than half. The results of production yield, affinity, stability measurements and analysis of three-dimensional models of the structure suggest that the sixth amino acid influences the correct folding of the V-H domain, presumably by affecting a folding intermediate, but has no effect on antigen binding.

L28 ANSWER 83 OF 128 MEDLINE DUPLICATE 28

97321108 Document Number: 97321108. PubMed ID: 9177839. **Single-chain Fv**/folate conjugates mediate efficient lysis of folate-receptor-positive tumor cells. Cho B K; Roy E J; Patrick T A; Kranz D M. (Department of Biochemistry, University of Illinois, Urbana 61801-3792, USA.) BIOCONJUGATE CHEMISTRY, (1997 May-Jun) 8 (3) 338-46. Journal code: 9010319. ISSN: 1043-1802. Pub. country: United States. Language: English.

AB **Bispecific** antibodies that bind to a tumor antigen and the T cell receptor (TCR) redirect cytotoxic T lymphocytes (CTL) to lyse tumor cells which have escaped normal immune recognition mechanisms. One well-characterized tumor antigen, the folate receptor (FR), is expressed on most ovarian carcinomas and some types of brain cancer. Recently, it was shown that conjugates of folate and anti-TCR antibodies are extremely potent **bispecific** agents that target tumor cells expressing the high-affinity folate receptor, but not normal cells expressing only the reduced folate carrier protein. In this paper, it is shown that the size of these conjugates can be reduced to the smallest **bispecific** agent yet described (30 kDa) by attaching folate to a single-chain antibody, scFv, of the anti-TCR antibody KJ16. The scFv/folate conjugates are as effective as IgG/folate conjugates in mediating lysis of FR4 tumor cells by CTL. The optimal folate density was in the range of 5-15 folate molecules per scFv or IgG molecule, which yielded half-maximal lysis values (EC50) of approximately 40 pM (1.2 ng/mL for scFv). Finally, the scFv/folate conjugates could efficiently target tumor cells even in the presence of free folic acid at concentrations that are normally found in serum. Compared to conventional **bispecific** antibodies, the small size of scFv/folate conjugates may prove advantageous in the ability to penetrate tumors and in reduced immunogenicity.

L28 ANSWER 84 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)

97:324922 The Genuine Article (R) Number: WV040. Antibody engineering. Hayden M S (Reprint); Gilliland L K; Ledbetter J A. BRISTOL MYERS SQUIBB PHARMACEUT RES INST, DEPT AUTOIMMUN & TRANSPLANTAT, 3005 1ST AVE, SEATTLE, WA 98121 (Reprint); UNIV OXFORD, SIR WILLIAM DUNN SCH PATHOL, OXFORD OX1

3RE, ENGLAND. CURRENT OPINION IN IMMUNOLOGY (APR 1997) Vol. 9, No. 2, pp. 201-212. Publisher: CURRENT BIOLOGY LTD. 34-42 CLEVELAND STREET, LONDON, ENGLAND W1P 6LB. ISSN: 0952-7915. Pub. country: USA; ENGLAND. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The development of recombinant techniques for the rapid cloning, expression, and characterization of cDNAs encoding antibody (Ab) subunits has revolutionized the field of antibody engineering. By fusion to heterologous protein domains, chain shuffling, and inclusion of self-assembly motifs, novel molecules such as **bispecific** Abs can now be generated which possess the subset of functional properties designed to fit the intended application. Rapid technological developments in phage display of peptides and proteins have led to a plethora of applications directed towards immunology and antibody engineering. Many of the problems associated with the therapeutic use of Abs are being addressed by the application of these new techniques.

L28 ANSWER 85 OF 128 MEDLINE DUPLICATE 29

1998098161 Document Number: 98098161. PubMed ID: 9435872. Construction and biological activity of a recombinant **bispecific** single-chain antibody designed for therapy of minimal residual colorectal cancer. Kufer P; Mack M; Gruber R; Lutterbuse R; Zettl F; Riethmuller G. (Institute of Immunology, University of Munich, Germany.) CANCER IMMUNOLOGY, IMMUNOTHERAPY, (1997 Nov-Dec) 45 (3-4) 193-7. Journal code: 8605732. ISSN: 0340-7004. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Unlike monoclonal antibodies, clinical application of **bispecific** antibodies has so far lagged behind because of difficult, low-yield production techniques as well as considerable toxicity attributed to **bispecific** antibody preparations containing immunoglobulin-Fc parts and anti-CD3 homodimers. These difficulties were overcome by recombinant generation of a **bispecific** single-chain antibody (bscAb) joining two **single-chain Fv** fragments via a five-amino-acid glycine-serine linker. The anti-CD3 specificity directed against human T cells was combined with another specificity against the epithelial 17-1A antigen. The following domain arrangement was critical in this individual case to obtain a fully functional bscAb: VL17-1A-VH17-1A-VHCD3-VLCD3. The bscAb was expressed in chinese hamster ovary cells with a yield of 15 mg/l culture supernatant whereas numerous attempts to obtain a functional protein expression in Escherichia coli failed. The low-molecular-mass **bispecific** construct (60 kDa) could easily be purified by its C-terminal histidine tail. The antigen-binding properties are indistinguishable from those of the corresponding univalent **single-chain Fv** fragments as shown by enzyme immunoassay and flow cytometry. We could show that the bscAb, which lacks Fc parts and anti-CD3 homodimers is highly cytotoxic for 17-1A positive tumor cells in nanomolar concentrations and in the presence of human T cells. Most remarkable, it does not stimulate T lymphocyte proliferation in the absence of tumor cells and, moreover, does not induce CD25 up-regulation and the secretion of potentially toxic lymphokines such as tumor necrosis factor alpha, interleukin-6 and interferon gamma. Maximal cytotoxicity (51Cr release) was achieved without notable costimulation and was not further enhanced by adding costimulatory signals such as those delivered by anti-CD28 antibodies. CD8+ as well as CD4+ T cell subpopulations were recruited to exert cytotoxicity against tumor cells with different kinetics. CD8+ cells induced high 51Cr release within 4 h while CD4+ cells required a 20-h incubation. The systemic application of the 17-1A/CD3-bscAb could be a major improvement in therapy against disseminated micrometastatic tumor cells. A prospective, randomized clinical trial showing that an anti-17-1A monoclonal antibody could prolong survival of colorectal cancer patients after 5 and 7 years, warrants an assessment of the clinical efficacy of this bscAb exhibiting a 1000-fold higher specific cytotoxicity against tumor cells in vitro.

L28 ANSWER 86 OF 128 CAPLUS COPYRIGHT 2002 ACS
1997:447493 Document No. 127:79887 Genetically engineered immunoglobulins and their application. Imai, Kozo; Kikuchi, Kokichi (Daiichi Naika, Sapporo Ika Daigaku, Sapporo, 060, Japan). Immunology Frontier, 7(3), 183-193 (Japanese) 1997. CODEN: IMFREG. ISSN: 0917-0774. Publisher: Medikaru Rebyusha.
AB A review with 31 refs., on recent progress in processing of monoclonal antibodies by genetic engineering and their application to gene therapy for tumors and autoimmune diseases, discussing establishment and characterization of chimeric monoclonal antibodies, CDR-grafted antibodies (humanized antibody), and recombinant antibodies including **single -chain Fv** antibody (ScFv), minibody, and **bispecific** ScFv.

L28 ANSWER 87 OF 128 MEDLINE DUPLICATE 30
1998098153 Document Number: 98098153. PubMed ID: 9435864.
Bispecific antibody treatment of murine B cell lymphoma. De Jonge J; Heirman C; De Veerman M; Van Meirvenne S; Demanet C; Brissinck J; Thielemens K. (Laboratory of Physiology, Medical School, Vrije Universiteit Brussel, (VUB), Belgium.) CANCER IMMUNOLOGY, IMMUNOTHERAPY, (1997 Nov-Dec) 45 (3-4) 162-5. Journal code: 8605732. ISSN: 0340-7004. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.
AB This report summarizes our experimental data concerning the use of **bispecific** antibodies (bsAb) for the treatment of the murine BCL1 B cell lymphoma model. Initially we used a hybrid-hybridoma-derived bsAb with specificity for the TcR/CD3 complex on T cells and the idiotype of the membrane-bound IgM on the tumor cells. The bsAb used as a single agent could cure animals with a low tumor load (resembling minimal residual disease). However, in experiments aimed at increasing the therapeutic effect in animals with a higher tumor burden, we could demonstrate the importance of additional T-cell-costimulatory signals and the careful timing of the bsAb administration. Recently we have generated a **bispecific single-chain Fv** (bsscFv) fusion protein with the same dual specificity as the hybrid-hybridoma-derived bsAb. Immunotherapy with this smaller molecule also resulted in tumor elimination in BCL1-bearing mice. A second bsscFv (alpha-CD19 x alpha-CD3) with a broader applicability is now being characterized and tested in vivo.

L28 ANSWER 88 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
97:115363 The Genuine Article (R) Number: WE998. Design and production of novel tetravalent **bispecific** antibodies. Coloma M J; Morrison S L (Reprint). UNIV CALIF LOS ANGELES, DEPT MICROBIOL & MOL GENET, 405 HILGARD AVE, LOS ANGELES, CA 90095 (Reprint); UNIV CALIF LOS ANGELES, DEPT MICROBIOL & MOL GENET, LOS ANGELES, CA 90095; UNIV CALIF LOS ANGELES, INST MOL BIOL, LOS ANGELES, CA 90095. NATURE BIOTECHNOLOGY (FEB 1997) Vol. 15, No. 2, pp. 159-163. Publisher: NATURE PUBLISHING CO. 345 PARK AVE SOUTH, NEW YORK, NY 10010-1707. ISSN: 1087-0156. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB We have produced novel **bispecific** antibodies by fusing the DNA encoding a single chain antibody (ScFv) after the C terminus (C(H)3-ScFv) or after the hinge (Hinge-ScFv) with an antibody of a different specificity, The fusion protein is expressed by gene transfection in the context of a murine variable region, Transfectomas secrete a homogeneous population of the recombinant antibody with two different specificities, one at the N terminus (anti-dextran) and one at the C terminus (anti-dansyl). The C(H)3-ScFv antibody, which maintains the constant region of human IgG3, has some of the associated effector functions such as long half-life and Fc receptor binding, The Hinge-ScFv antibody which lacks the C(H)2 and C(H)3 domains has no known effector functions.

L28 ANSWER 89 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
97:542545 The Genuine Article (R) Number: XK773. New protein engineering approaches to multivalent and **bispecific** antibody fragments. Pluckthun A (Reprint); Pack P. UNIV ZURICH, INST BIOCHEM, CH-8057 ZURICH, SWITZERLAND; MORPHOSYS GMBH, D-80807 MUNICH, GERMANY. IMMUNOTECHNOLOGY (JUN 1997) Vol. 3, No. 2, pp. 83-105. Publisher: ELSEVIER SCIENCE BV. PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 1380-2933. Pub. country: SWITZERLAND; GERMANY. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Multivalency is one of the hallmarks of antibodies, by which enormous gains in functional affinity, and thereby improved performance in vivo and in a variety of in vitro assays are achieved. Improved in vivo targeting and more selective localization are another consequence of multivalency. We summarize recent progress in engineering multivalency from recombinant antibody fragments by using miniantibodies (scFv fragments linked with hinges and oligomerization domains), spontaneous scFv dimers with short linkers (diabodies), or chemically crosslinked antibody fragments. Directly related to this are efforts of bringing different binding sites together to create **bispecific** antibodies. For this purpose, chemically linked fragments, diabodies, scFv-scFv tandems and **bispecific** miniantibodies have been investigated. Progress in E. coli expression technology makes the amounts necessary for clinical studies now available for suitably engineered fragments. We foresee therapeutic advances from a modular, systematic approach to optimizing pharmacokinetics, stability and functional affinity, which should prove possible with the new recombinant molecular designs. (C) 1997 Elsevier Science B.V.

L28 ANSWER 90 OF 128 CAPLUS COPYRIGHT 2002 ACS
1996:198737 Document No. 124:258003 Leucine zipper dimerized bivalent and **bispecific** scFv antibodies from a semi-synthetic antibody phage display library. de Kruif, John; Logtenberg, Ton (Dep. Immunology, Utrecht Univ., Utrecht, 3508 GA, Neth.). Journal of Biological Chemistry, 271(13), 7630-4 (English) 1996. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB This report describes the construction of leucine zipper-based dimerization cassettes for the conversion of recombinant monomeric scFv antibody fragments to bivalent and **bispecific** dimers. A truncated murine IgG3 hinge region and a Fos or Jun leucine zipper were cloned into four scFv fragments previously isolated from a synthetic antibody phage display library. Cysteine residues flanking the zipper region were introduced to covalently.link dimerized scFv fragments. The secreted fusion proteins were shown to spontaneously and efficiently form stable Fos.cntdot.Fos or Jun.cntdot.Jun homodimers in the Escherichia coli periplasm at levels comparable to their monovalent counterparts. The bivalent (scFv)2 fragments performed well in ELISA, flow-cytometric, and immunohistochem. anal. Fos and Jun homodimer (scFv)2 antibodies with different specificities could be reduced, reshuffled, and reoxidized to form prepns. of functional **bispecific** (scFv)2 Fos.cntdot.Jun heterodimers. These Fos and Jun fusion protein cassettes provide a universal basis for the construction of dimeric scFv antibodies with enhanced avidity or dual specificity.

L28 ANSWER 91 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
96:475433 The Genuine Article (R) Number: UT398. MINIBODY - A NOVEL ENGINEERED ANTICARCINOEMBRYONIC ANTIGEN-ANTIBODY FRAGMENT (**SINGLE -CHAIN FV**-C(H)3) WHICH EXHIBITS RAPID, HIGH-LEVEL TARGETING OF XENOGRAFTS. HU S Z; SHIVERY L; RAUBITSCHEK A; SHERMAN M; WILLIAMS L E; WONG J Y C; SHIVELY J E; WU A M (Reprint). CITY HOPE NATL MED CTR, BECKMAN RES INST, DEPT BIOL MOLEC, 1450 E DUARTE RD, DUARTE, CA, 91010 (Reprint); CITY HOPE NATL MED CTR, BECKMAN RES INST, DEPT BIOL MOLEC, DUARTE, CA, 91010; CITY HOPE NATL MED CTR, BECKMAN RES INST, DIV

BIOL, DUARTE, CA, 91010; CITY HOPE NATL MED CTR, BECKMAN RES INST, DIV IMMUNOL, DUARTE, CA, 91010; CITY HOPE NATL MED CTR, BECKMAN RES INST, DIV DIAGNOST RADIOL, DUARTE, CA, 91010; CITY HOPE NATL MED CTR, BECKMAN RES INST, DIV RADIAT ONCOL, DUARTE, CA, 91010; CITY HOPE NATL MED CTR, BECKMAN RES INST, DEPT RADIOIMMUNOTHERAPY, DUARTE, CA, 91010. CANCER RESEARCH (01 JUL 1996) Vol. 56, No. 13, pp. 3055-3061. ISSN: 0008-5472. Pub. country: USA. Language: ENGLISH.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A novel engineered antibody fragment (V-L-V-H-C(H)3, or ''minibody'') with bivalent binding to carcinoembryonic antigen (CEA) was produced by genetic fusion of a T84.66 (anti-CFA) single-chain antibody (scFv) to the human IgG1 C(H)3 domain. Two designs for the connecting peptide were evaluated. In the T84.66/212 LD minibody, a two-amino acid linker (generated by fusion of restriction sites) was used to join V-H and C(H)3. In the T84.66/212 Flex minibody, the human IgG1 hinge plus an additional 10 residues were used as the connecting peptide. Size exclusion chromatography of purified minibodies demonstrated that both proteins had assembled into M(r)80,000 dimers as expected. Furthermore, analysis by SDS-PAGE under nonreducing conditions was consistent with disulfide bond formation in the hinge of the T84.66 Flex minibody. Purified minibodies retained high affinity for CEA (K-A, 2 x 10(9) M(-1)) and demonstrated bivalent binding to antigen. Tumor targeting properties were evaluated in vivo using athymic mice bearing LS174T human colon carcinoma xenografts. I-123-labeled T84.66 minibodies demonstrated rapid, high tumor uptake, reaching 17% injected dose/gram (%ID/g) for the LD minibody and 33%ID/g for the Flex minibody at 6 h following injection. Radioiodinated minibody also cleared rapidly from the circulation, yielding high tumor:blood uptake ratios: 44.5 at 24 h for the LD minibody and 64.9 at 48 h for the Flex minibody. Rapid localization by the T84.66/212 Flex minibody allowed imaging of xenografts at 4 and 19 h after administration.

L28 ANSWER 92 OF 128 MEDLINE DUPLICATE 31

97044103 Document Number: 97044103. PubMed ID: 8889174. Symmetry of Fv architecture is conducive to grafting a second antibody binding site in the Fv region. Keck P C; Huston J S. (Creative BioMolecules, Hopkinton, Massachusetts 01748, USA.) BIOPHYSICAL JOURNAL, (1996 Oct) 71 (4) 2002-11. Journal code: 0370626. ISSN: 0006-3495. Pub. country: United States. Language: English.

AB Molecular modeling studies on antibody Fv regions have been pursued to design a second antigen-binding site (chi-site) in a chimeric **single-chain Fv** (chi sFv) species of about 30 kDa. This analysis has uncovered an architectural basis common to many Fv regions that permits grafting a chi-site onto the Fv surface that diametrically opposes the normal combining site. By using molecular graphics analysis, chimeric complementarity-determining regions (chi CDRs) were defined that comprised most of the CDRs from an antibody binding site of interest. The chain directionality of chi CDRs was consistent with that of specific bottom loops of the sFv, which allowed for grafting of chi CDRs with an overall geometry approximating CDRs in the parent combining site. Analysis of 10 different Fv crystal structures indicates that the positions for inserting chi CDRs are very highly conserved, as are the corresponding chi CDR boundaries in the parent binding site. The results of this investigation suggest that it should be possible to generally apply this approach to the development of chimeric **bispecific** antibody binding site (chi BABS) proteins.

L28 ANSWER 93 OF 128 MEDLINE DUPLICATE 32

96424326 Document Number: 96424326. PubMed ID: 8826849. Targeted inhibition of tumour cell growth by a **bispecific** single-chain toxin containing an antibody domain and TGF alpha. Schmidt M; Wels W. (Institute for Experimental Cancer Research, Freiburg, Germany.) BRITISH JOURNAL OF CANCER, (1996 Sep) 74 (6) 853-62. Journal code: 0370635. ISSN: 0007-0920. Pub. country: SCOTLAND: United Kingdom. Language: English.

AB Overexpression of the epidermal growth factor receptor (EGFR) and ErbB-2 has been observed in a variety of human tumours, making these receptors promising targets for directed tumour therapy. Since many tumour cells express both ErbB-2 and EGFR and these receptors synergise in cellular transformation, therapeutic reagents simultaneously binding to ErbB-2 and EGFR might offer advantages for tumour therapy. We have previously described the potent anti-tumoral activity of a **bispecific** antibody toxin that contains ErbB-2- and EGFR-specific **single-chain Fv** (scFv) domains. Here we report the construction and functional characterisation of a novel **bispecific** recombinant toxin, scFv(FRP5)-TGF alpha-ETA. The fusion protein consists of the antigen-binding domain of the ErbB-2-specific MAb, FRP5, and the natural EGFR ligand, TGF alpha, inserted at different positions in truncated Pseudomonas exotoxin A. ScFv(FRP5)-TGF alpha-ETA protein displayed binding to EGFR and ErbB-2, thereby inducing activation of the receptors, which was dependent on the cellular context and the level of EGFR and ErbB-2 expression. The **bispecific** molecule was cytotoxic in vitro for tumour cells expressing various levels of the target receptors. In vivo scFv(FRP5)-TGF alpha-ETA potently inhibited the growth of established A431 tumour xenografts in nude mice.

L28 ANSWER 94 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1996:257318 Document No.: PREV199698813447. Production of **bispecific single-chain Fvs** (sFV')-2 specific for the oncogene product c-erbB-2 and human CD16/mouse Fc-gamma-RII/III using recombinant phage display libraries. McCall, A. M. (1); Schier, R.; Amoroso, A. (1); Sautes, C.; Adams, G. P. (1); Marks, J. D.; Weiner, L. M. (1). (1) Fox Chase Cancer Cent., Philadelphia, PA 19111 USA. Proceedings of the American Association for Cancer Research Annual Meeting, (1996) Vol. 37, No. 0, pp. 472. Meeting Info.: 87th Annual Meeting of the American Association for Cancer Research Washington, D.C., USA April 20-24, 1996 ISSN: 0197-016X. Language: English.

L28 ANSWER 95 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1996:257317 Document No.: PREV199698813446. Influence of avidity on the tumor retention of monospecific and **bispecific** anti-c-erbB-2 **single-chain FV** dimers. Adams, G. P. (1); McCartney, J. E.; Wolf, E. J. (1); Tai, M.-S.; Schier, R.; Stafford, W. F.; Marks, J. D.; Bookman, M. A. (1); Huston, J. S.; Weiner, L. M. (1). (1) Fox Chase Cancer Cent., Phildelphia, PA 19111 USA. Proceedings of the American Association for Cancer Research Annual Meeting, (1996) Vol. 37, No. 0, pp. 472. Meeting Info.: 87th Annual Meeting of the American Association for Cancer Research Washington, D.C., USA April 20-24, 1996 ISSN: 0197-016X. Language: English.

L28 ANSWER 96 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
96:475243 The Genuine Article (R) Number: UR722. ADVANCES IN ANTIBODY ENGINEERING. GEORGE A J T (Reprint); EPENETOS A A. HAMMERSMITH HOSP, ROYAL POSTGRAD MED SCH, DEPT IMMUNOL, DU CANE RD, LONDON W12 0NN, ENGLAND (Reprint); ANTISOMA LTD, LONDON W5 3QR, ENGLAND. EXPERT OPINION ON THERAPEUTIC PATENTS (MAY 1996) Vol. 6, No. 5, pp. 441-456. ISSN: 1354-3776 . Pub. country: ENGLAND. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Our ability to genetically modify antibody molecules has been dramatically enhanced by a number of technological breakthroughs, including the ability to rapidly and easily clone the variable region genes, and the development of good expression systems. in addition phage display technology has given a new impetus to the field by allowing the isolation and modification of novel antibodies. This paper reviews these important areas, and describes some of the more common genetic constructs that have been produced. Such recombinant molecules are entering clinical trials more frequently and will become increasingly important as diagnostic and therapeutic agents.

L28 ANSWER 97 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
96:903818 The Genuine Article (R) Number: BG71H. Antibody binding sites. Huston J S (Reprint); Margolies M N; Haber E. CREAT BIOMOL INC, HOPKINTON, MA 01748 (Reprint); MASSACHUSETTS GEN HOSP, DEPT SURG, BOSTON, MA 02114; HARVARD UNIV, SCH MED, BOSTON, MA 02114; HARVARD UNIV, SCH MED, DEPT MED, BOSTON, MA 02115; HARVARD UNIV, SCH PUBL HLTH, CARDIOVASC BIOL LAB, BOSTON, MA 02115. ANTIGEN BINDING MOLECULES: ANTIBODIES AND T-CELL RECEPTORS (8 NOV 1996) Vol. 49, pp. 329-450. Publisher: ACADEMIC PRESS INC . 525 B STREET, SUITE 1900, SAN DIEGO, CA 92101-4495. ISSN: 0065-3233. Pub. country: USA. Language: English.

L28 ANSWER 98 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1997:139160 Document No.: PREV199799438363. Leucine zipper dimerized bivalent and **bispecific** SCFV antibodies from a phage display library. De Kruif, John; Logtenberg, Ton. Utrecht Univ. Hosp., Dep. Immunol., PO Box 85500, 3508GA Utrecht Netherlands. Immunotechnology (Amsterdam), (1996) Vol. 2, No. 4, pp. 298-299. Meeting Info.: 1996 Keystone Meeting on Exploring and Exploiting Antibody and Ig Superfamily Combining Sites Taos, New Mexico, USA February 22-28, 1996 ISSN: 1380-2933. Language: English.

L28 ANSWER 99 OF 128 CAPLUS COPYRIGHT 2002 ACS
1996:719647 Document No. 126:6120 Costimulation by CD28 sFv expressed on the tumor cell surface or as a soluble **bispecific** molecule targeted to the L6 carcinoma antigen. Hayden, M. S.; Grosmaire, L. S.; Norris, N. A.; Gilliland, L. K.; Winberg, G.; Tritschler, D.; Tsu, T. T.; Linsley, P. S.; Mittler, R. S.; et al. (Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA, 98121, USA). Tissue Antigens, 48(4-I), 242-254 (English) 1996. CODEN: TSANA2. ISSN: 0001-2815. Publisher: Munksgaard.
AB Interaction of the CD80 (B7-1) and CD86 (B7-2) mols. on antigen presenting cells with the receptors CD28 and CTLA-4 on T cells generates signals important in the regulation of immune responses. Because this receptor system involves multiple receptor-ligand interactions, detg. the function for individual receptors has been difficult. One approach is the use of antibodies and their derivs. with singular specificity as substitute ligands to explore the activities of these mols. The authors constructed recombinant mono- and **bispecific** sFv mols. specific for the CD28 receptor that are capable of binding and generating costimulatory signals to activate T cells. They demonstrate that these sol. mols. are capable of higher levels of costimulation than sol. CD80Ig at equiv. concns. They also constructed artificial adhesion receptors on the cell surface using two different CD28-specific sFvIgs fused to the CD80 cytoplasmic and transmembrane domains. Here, the authors compared costimulation by a sol. **bispecific** (.alpha.CD28-.alpha.L6) single chain sFvIg fusion protein to that generated by L6 antigen pos. (L6+) H3347 tumor cells transduced with cell surface expressed forms of .alpha.CD28 sFv. The authors show that the **bispecific** protein can target potent CD28 costimulatory activity to L6+ tumor cells in vitro. They also show that transfection of the cell surface forms of the two different CD28 sFvIgs into H3347 tumor cells allows them to generate costimulatory signals to activated T cells. Finally, it is demonstrated that tumor cell presentation of either the sol. **bispecific** or transduced cell surface sFv generate similar costimulatory effects resulting in T cell activation.

L28 ANSWER 100 OF 128 MEDLINE
97044732 Document Number: 97044732. PubMed ID: 8889803. Construction and expression of bi-functional proteins of **single-chain Fv** with effector domains. Luo D; Mah N; Wishart D; Zhang Y; Jacobs F; Martin L. (Research and Development Division, Biomira Inc., Edmonton, Alberta, Canada.) JOURNAL OF BIOCHEMISTRY, (1996 Aug) 120 (2) 229-32. Journal code: 0376600. ISSN: 0021-924X. Pub. country: Japan. Language: English.

AB We fused various polypeptide extensions to the C-termini of **single chain Fv** (scFv) and disulfide-stabilized Fv (dsFv) fragments to facilitate detection of bi-functional proteins or to add biological effector domains, which included the human metallothionein (HMT) motif and biotin mimetic sequence. These bi-functional proteins were expressed and secreted in a recombinant Pichia pastoris system and showed specific anti-idiotype binding activity, as determined by competitive radioimmunoassaying. However, the fusion protein constructed with dsFv-HMT, but not scFv-HMT, had lost this binding activity. The interruption of the structural conformation as a result in dsFv-HMT may be explained by the interactions between the cysteines engineered in dsFv domains and the cysteines in the HMT region.

L28 ANSWER 101 OF 128 MEDLINE DUPLICATE 33
96215470 Document Number: 96215470. PubMed ID: 8649442. A single-chain **bispecific** Fv2 molecule produced in mammalian cells redirects lysis by activated CTL. Jost C R; Titus J A; Kurucz I; Segal D M. (Experimental Immunology Branch, National Cancer Institute, Bethesda, MD 20892, USA.) MOLECULAR IMMUNOLOGY, (1996 Feb) 33 (2) 211-9. Journal code: 7905289. ISSN: 0161-5890. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **Single-chain Fv** (sFv) molecules consist of the two variable domains of an antibody (Ab) connected by a polypeptide spacer and contain the binding activities of their parental antibodies (Abs). In this paper we have attached the C-terminus of 2C11-sFv (anti-mouse CD3 epsilon-chain) to the N-terminus of OKT9-sFv (anti-human transferrin receptor [TfR]) through a 23 amino acid inter-sFv linker consisting primarily of CH1 region residues from 2C11, to form a single-chain **bispecific** Fv2 [bs(sFv)2] molecule. The bs(sFv)2 was expressed in COS-7 cells, and was secreted at the same rate as the two parental sFvs. The secreted protein had both anti-CD3 and anti-TfR binding activities. Essentially all of the secreted bs(sFv)2 molecules bound TfR and the binding affinity of the bs(sFv)2 was comparable to that of OKT9 sFv and Fab. Thus, the attachment of the inter-sFv linker to the N-terminus of OKT9-sFv did not impair its binding function. The bs(sFv)2 retained both binding specificities after long-term storage at 4 degrees C or overnight incubation at 37 degrees C. It redirected activated mouse CTL to specifically lyse human TfR+ target cells at low (ng/ml) concentrations and was much more active than a chemically cross-linked heteroconjugate prepared from the same parental mAbs. Because bs(sFv)2 molecules secreted by mammalian cells are homogeneous proteins containing two binding sites in a single polypeptide chain, they hold great promise as an easily obtainable, economic source of a **bispecific** molecule suitable for in vivo use.

L28 ANSWER 102 OF 128 CAPLUS COPYRIGHT 2002 ACS
1996:709425 Document No. 125:325550 Targeting of peripheral blood T lymphocytes. Bolhuis, Reinder L. H.; Hoogenboom, Hennie R.; Gratama, Jan Willem (Department Clinical and Tumor Immunology, Daniel den Hoed Cancer Center, Rotterdam, 3075 EA, Neth.). Springer Seminars in Immunopathology, 18(2), 211-226 (English) 1996. CODEN: SSIMDV. ISSN: 0344-4325. Publisher: Springer.

AB A review with 123 refs. first describing the T-cell and target cell structures that are well known to play major roles in effector lymphocyte/target cell interactions and then focusing on: (1) prodn. of **bispecific** monoclonal antibodies and **single-chain Fv** monoclonal antibodies for targeting of T-cells; (2) targeting of T-cells with **bispecific** monoclonal antibodies; and (3) targeting of T-cells by genetically engineering single-chain Ig/.gamma. or .zeta. receptors into T-cells.

L28 ANSWER 103 OF 128 MEDLINE DUPLICATE 34
97110906 Document Number: 97110906. PubMed ID: 9005442. Affinity

enhancement of a recombinant antibody: formation of complexes with multiple valency by a **single-chain Fv** fragment-core streptavidin fusion. Kipriyanov S M; Little M; Kropshofer H; Breitling F; Gotter S; Dubel S. (Recombinant Antibody Research Group, Heidelberg, Germany.) PROTEIN ENGINEERING, (1996 Feb) 9 (2) 203-11. Journal code: 8801484. ISSN: 0269-2139. Pub. country: ENGLAND: United Kingdom. Language: English.

AB In antigen-antibody interactions, the high avidity of antibodies depends on the affinity and number of the individual binding sites. To develop artificial antibodies with multiple valency, we have fused the single-chain antibody Fv fragments to core streptavidin. The resulting fusion protein, termed scFv::strep, was found after expression in Escherichia coli in periplasmic inclusion bodies. After purification of the recombinant product by immobilized metal affinity chromatography, refolding and size-exclusion FPLC, tetrameric complexes resembling those of mature streptavidin were formed. The purified tetrameric scFv::strep complexes demonstrated both antigen- and biotin-binding activity, were stable over a wide range of pH and did not dissociate at high temperatures (up to 70 degrees C). Surface plasmon resonance measurements in a BIAlite system showed that the pure scFv::strep tetramers bound immobilized antigen very tightly and no dissociation was measurable. The association rate constant for scFv::strep tetramers was higher than those for scFv monomers and dimers. This was also reflected in the apparent constants, which was found to be 35 times higher for pure scFv::strep tetramers than monomeric single-chain antibodies. We could also show that most of biotin binding sites were accessible and not blocked by biotinylated E.coli proteins or free biotin from the medium. These sites should therefore facilitate the construction of **bispecific** multivalent antibodies by the addition of biotinylated ligands.

L28 ANSWER 104 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)

96:37283 The Genuine Article (R) Number: TM481. BINDING CHARACTERISTICS AND ANTITUMOR PROPERTIES OF 1A10 **BISPECIFIC** ANTIBODY RECOGNIZING GP40 AND HUMAN TRANSFERRIN RECEPTOR. AMOROSO A R; CLARK J I; LITWIN S; HSIEHMA S; SHI T; ALPAUGH R K; ADAMS G P; WOLF E J; RING D B; WEINER L M (Reprint). FOX CHASE CANC CTR, DEPT MED ONCOL, 7701 BURHOLME AVE, PHILADELPHIA, PA, 19131 (Reprint); FOX CHASE CANC CTR, DEPT MED ONCOL, PHILADELPHIA, PA, 19131; FOX CHASE CANC CTR, DEPT BIOSTAT, PHILADELPHIA, PA, 19131; CHIRON CORP, EMERYVILLE, CA, 94608. CANCER RESEARCH (01 JAN 1996) Vol. 56, No. 1, pp. 113-120. ISSN: 0008-5472. Pub. country: USA. Language: ENGLISH.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The **bispecific** murine monoclonal antibody (MAb) 1A10 has specificity for the human transferrin receptor (TfR) and the human tumor-associated antigen gp40. This antibody, therefore, functions as an ''antigen fork'' by binding to two distinct antigens on the same malignant cell. Highly purified 1A10 inhibits the growth of cells coexpressing high levels of human TfR and the tumor-associated antigen gp40 by binding to both target antigens. In SW948 cells, the majority of 1A10 binding is via its gp40 specificity, and half-maximal inhibition of cell growth by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyletrazolium bromide assay requires 20-30-mu g/ml concentrations of 1A10. The binding of 1A10 correlates with growth inhibition in the cell lines HT-29, SK-OV-3, OVCAR-2, and OVCAR-3. The growth of OVCAR-10 cells, which express little gp40 and TfR, is not inhibited by 1A10. However, SK-BR3 cells, which express abundant gp40 and extremely high levels of TfR, are insensitive to the effects of 1A10. In some cell lines, combined exposure to 1A10 and the iron chelator deferoxamine mesylate has synergistic antiproliferative effects. A single i.p. dose of 600 mu g 1A10 is sufficient to achieve an estimated tumor concentration of at least 30 mu g/ml for 7 days in C.B17/Icr-scid mice bearing SW948 human tumor xenografts. Treatment of scid mice bearing day 2 or day 4 SW948 xenografts with single or multiple 1A10 doses inhibits tumor growth in a dose-related fashion. Antitumor effects are not seen

with therapy using either parental antibody of 1A10. The antiproliferative properties of 1A10 in tumor cells overexpressing gp40 and TfR suggest avenues for the development of new **bispecific** antibody-promoted treatment strategies.

L28 ANSWER 105 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
96:316230 The Genuine Article (R) Number: UF477. TUMOR-LOCALIZATION OF ANTI-CEA **SINGLE-CHAIN FVS** - IMPROVED TARGETING BY NONCOVALENT DIMERS. WU A M (Reprint); CHEN W G; RAUBITSCHEK A; WILLIAMS L E; NEUMAIER M; FISCHER R; HU S Z; ODOMMARYON T; WONG J Y C; SHIVELY J E. CITY HOPE NATL MED CTR, BECKMAN RES INST, DEPT MOLEC BIOCHEM, DUARTE, CA, 91010; CITY HOPE NATL MED CTR, DIV RADIAT ONCOL, DUARTE, CA, 91010; CITY HOPE NATL MED CTR, DIV RADIOL, DUARTE, CA, 91010; CITY HOPE NATL MED CTR, DEPT BIOSTAT, DUARTE, CA, 91010; CITY HOPE NATL MED CTR, BECKMAN RES INST, DIV IMMUNOL, DUARTE, CA, 91010; UNIV HAMBURG HOSP, ABT KLIN CHEM, HAMBURG, GERMANY. IMMUNOTECHNOLOGY (FEB 1996) Vol. 2, No. 1, pp. 21-36. ISSN: 1380-2933. Pub. country: USA; GERMANY. Language: ENGLISH. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Background: Genetic engineering can produce novel antibody fragments with improved properties for applications such as tumor targeting in vivo. Objectives: To produce stable monomeric (27 kDa) and dimeric (55 kDa) forms of a **single-chain Fv** (scFv) from the anti-carcinoembryonic antigen (anti-CEA) antibody T84.66, and assess the targeting and biodistribution properties in an animal model. Study design: ScFv were constructed with either a 28 or 14 amino acid connecting peptide and expressed by secretion from E. coli. Following affinity purification, proteins were characterized by gel electrophoresis and mass spectrometry. Binding properties were assessed by size exclusion HPLC after incubation with antigen, and affinities determined by surface plasmon resonance. The shorter linker favored formation of dimers (and higher multimers) which showed unusual stability. ScFv were radiolabeled with I-125 for tumor targeting and biodistribution studies of monomeric or dimeric forms were conducted in athymic mice bearing LS174T human colorectal carcinoma xenografts. Results: I-125-sCFv monomers and dimers targeted exhibited rapid clearance kinetics in tumor-bearing mice. Nevertheless, the anti-CEA scFvs targeted very well to xenografts, leading to high tumor: normal organ ratios (greater than 20:1 at 24 h) for both forms. Tumor localization of the non-covalent dimers was much higher than monomers, reaching 10-15% injected dose per gram at 1 h. Conclusion: Non-covalent dimers of scFv (also known as diabodies) are stable, easy to produce and show excellent targeting as compared to monomeric scFv, probably due to increased mass and valency.

L28 ANSWER 106 OF 128 MEDLINE DUPLICATE 35
95350203 Document Number: 95350203. PubMed ID: 7624362. A small **bispecific** antibody construct expressed as a functional single-chain molecule with high tumor cell cytotoxicity. Mack M; Riethmuller G; Kufer P. (Institut fur Immunologie, Munich, Germany.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1995 Jul 18) 92 (15) 7021-5. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Construction of a **bispecific** single-chain antibody derivative is described that consists of two different **single-chain Fv** fragments joined through a Gly-Ser linker. One specificity of the two Fv fragments is directed against the CD3 antigen of human T cells and the other is directed against the epithelial 17-1A antigen; the latter had been found in a clinical trial to be a suitable target for antibody therapy of minimal residual colorectal cancer. The construct could be expressed in CHO cells as a fully functional protein, while its periplasmic expression in Escherichia coli resulted in a nonfunctional protein only. The antigen-binding properties of the **bispecific** single-chain antibody are indistinguishable from those of the corresponding univalent **single-chain Fv**

fragments. By redirecting human peripheral T lymphocytes against 17-1A-positive tumor cells, the **bispecific** antibody proved to be highly cytotoxic at nanomolar concentrations as demonstrated by 51Cr release assay on various cell lines. The described **bispecific** construct has a molecular mass of 60 kDa and can be easily purified by its C-terminal histidine tail on a Ni-NTA chromatography column. As **bispecific** antibodies have already been shown to be effective in vivo in experimental tumor systems as well as in phase-one clinical trials, the small CD3/17-1A-**bispecific** antibody may be more efficacious than intact antibodies against minimal residual cancer cells.

L28 ANSWER 107 OF 128 MEDLINE DUPLICATE 36
96075435 Document Number: 96075435. PubMed ID: 7493381. Targeting c-erbB-2 expressing tumors using **single-chain Fv** monomers and dimers. Tai M S; McCartney J E; Adams G P; Jin D; Hudziak R M; Oppermann H; Laminet A A; Bookman M A; Wolf E J; Liu S; +. (Creative BioMolecules, Inc., Hopkinton, Massachusetts 01748, USA.) CANCER RESEARCH, (1995 Dec 1) 55 (23 Suppl) 5983s-5989s. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB **Single-chain Fv** proteins containing a COOH-terminal cysteine (sFv') were constructed by using an antidigoxin 26.10 sFv and an anti-c-erbB-2 741F8 sFv. The fully active sFv' proteins were prepared by expression in Escherichia coli as insoluble inclusion bodies, followed by in vitro refolding using glutathione redox buffers and purification. The COOH-terminal cysteines of the refolded sFv' proteins were protected by a blocking group presumed to be the glutathionyl peptide, which was easily and selectively removed by gentle reduction. Air oxidation of the reduced sFv' monomers resulted in the efficient formation of disulfide-linked sFv' homodimers, designated (sFv')2, which were stable under oxidizing conditions and relatively slow to be disrupted under reducing conditions. The (26-10-1 sFv')-(741F8-1 sFv') heterodimer was prepared and possessed dual-antigen specificity; the active bispecific (sFv')**2** dimerized under native conditions, apparently as a manifestation of self-association by the 741F8 sFv' subunit. Biodistribution and imaging studies that were performed on mice bearing human SK-OV-3 tumor xenografts that express the c-erbB-2 as a cell surface antigen were reviewed. Radioiodinated 741F8-2 (sFv')2 homodimer localized to the tumors with high specificity, as evidenced by excellent tumor:normal tissue ratios. Sagittal section autoradiography of whole animals 24 h after administration of antibody species revealed that 741F8 (sFv')2 produced a stronger tumor image than comparable doses of the 741F8 Fab, monomeric sFv', and the 26-10 (sFv')2 control without the high nonspecific background distribution of the 741F8 IgG.

L28 ANSWER 108 OF 128 CAPLUS COPYRIGHT 2002 ACS
1995:969306 Document No. 124:80946 Targeting c-erbB-2 expressing tumors using **single-chain Fv** monomers and dimers.
Tai, Mei-Sheng; McCartney, John E.; Adams, Gregory P.; Jin, Donald; Hudziak, Robert M.; Oppermann, Hermann; Laminet, Axel A.; Bookman, Michael A.; Wolf, Ellen J.; et al. (Creative BioMolecules, Inc., Hopkinton, MA, 01748, USA). Cancer Research, 55(23, Suppl.), 5983S-9S (English) 1995. CODEN: CNREA8. ISSN: 0008-5472. Publisher: American Association for Cancer Research.

AB **Single-chain Fv** proteins contg. a COOH-terminal cysteine (sFv') were constructed by using an antidigoxin 26-10 sFv and an anti-c-erbB-2 741F8 sFv. The fully active sFv' proteins were prepd. by expression in Escherichia coli as insol. inclusion bodies, followed by in vitro refolding using glutathione redox buffers and purifn. The COOH-terminal cysteines of the refolded sFv' proteins were protected by a blocking group presumed to be the glutathionyl peptide, which was easily and selectively removed in the efficient formation of disulfide-linked sFv' homodimers, designed (sFv')2, which were stable under oxidizing conditions and relatively slow to be disrupted under

reducing conditions. The (26-10-1 sFv')-(741F8-1 sFv') heterodimer was prepd. and possessed dual-antigen specificity; the active **bispecific** (sFv')2 dimerized under native conditions, apparently as a manifestation of self-assocn. by the 741F8 sFv' subunit. Biodistribution and imaging studies that were performed on mice bearing human SK-OV-3 tumor xenografts that express the c-erbB-2 as a cell surface antigen were reviewed. Radioiodinated 741F8-2 (sFv')2 homodimer localized to the tumors with high specificity, as evidenced by excellent tumor:normal tissue ratios. Sagittal section autoradiog. of whole animals 24 h after administration of antibody species revealed that 741F8 (sFv')2 produced a stronger tumor image than comparable doses of the 741F8 Fab, monomeric sFv', and the 26-10 (sFv')2 control without the high nonspecific background distribution of the 741F8 IgG.

L28 ANSWER 109 OF 128 MEDLINE DUPLICATE 37

95238955 Document Number: 95238955. PubMed ID: 7536774. Retargeting of CTL by an efficiently refolded **bispecific single-chain Fv** dimer produced in bacteria. Kurucz I; Titus J A; Jost C R; Jacobus C M; Segal D M. (Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA.) JOURNAL OF IMMUNOLOGY, (1995 May 1) 154 (9) 4576-82. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB A single-chain **bispecific** Fv dimer (bs(sFv)2) having specificity for mouse CD3 epsilon chain and human transferrin receptor was produced in bacterial inclusion bodies. To overcome difficulties associated with in vitro protein folding, we used a novel renaturation approach to obtain active bs(sFv)2. The protein was dissolved in the weak ionic detergent sodium lauroylsarcosine, and disulfides were formed by oxidation in air. After oxidation, the bs(sFv)2 exhibited very little covalent aggregation and migrated as a single species in nonreducing SDS-PAGE, suggesting that disulfides were correctly paired. The detergent was removed using an ion exchange resin and the protein fractionated by size exclusion chromatography. The recovered 65-kDa protein was monomeric in non-denaturing solvent, homogeneous by SDS-PAGE, and comprised 15 to 20% of material applied to the gel filtration column. This protein bound specifically to both mouse CD3 epsilon chain and human transferrin receptor with affinities indistinguishable from those of the parental Fabs or **single-chain Fvs**. The bs(sFv)2 specifically redirected mouse cytotoxic T cells to lyse target cells expressing human transferrin receptor at picomolar concentrations. Bacterially produced and detergent oxidized bs(sFv)2 molecules may therefore provide the abundant amounts of homogeneous active material required to redirect cytotoxic cells against tumors and other unwanted cells in animal models and in patients.

L28 ANSWER 110 OF 128 MEDLINE DUPLICATE 38

96256891 Document Number: 96256891. PubMed ID: 8643110. Production and characterization of **bispecific** single-chain antibody fragments. De Jonge J; Brissinck J; Heirman C; Demanet C; Leo O; Moser M; Thielemans K. (Laboratory of Physiology, Medical School, Vrije Universiteit, Brussels, Belgium.) MOLECULAR IMMUNOLOGY, (1995 Dec) 32 (17-18) 1405-12. Journal code: 7905289. ISSN: 0161-5890. Pub. country: ENGLAND: United Kingdom. Language: English.

AB We report the construction, expression and purification of a **bispecific single-chain Fv** antibody fragment produced in Escherichia coli. The protein possesses a dual specificity: the single-chain FvB1 portion is directed to the Idiotype of BCL1 lymphoma cells, the single-chain Fv2C11 moiety binds to the CD3 marker on T cells. The two domains are joined by a flexible peptide linker. Using Immobilized Metal Affinity Chromatography, the recombinant protein was purified from bacterial insoluble membrane fractions. After refolding of the **bispecific** protein, it was affinity-purified.

As demonstrated by flow cytometry, both binding sites are retained in the refolded protein. Retargeted cytotoxicity and T cell proliferation assays further prove the biological activity and specificity of the **bispecific single-chain Fv**. Thus, these **bispecific** molecules show a potential anti-tumor activity.

L28 ANSWER 111 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
95:784453 The Genuine Article (R) Number: TD752. RAPID ASSAY OF PHAGE-DERIVED RECOMBINANT HUMAN FABS AS **BISPECIFIC** ANTIBODIES. SANNA P P; DELOGU A; WILLIAMSON R A (Reprint); SAMSON M E; ALTIERI D C; BLOOM F E; BURTON D R. SCRIPPS CLIN & RES FDN, RES INST, DEPT NEUROPHARMACOL, 10666 N TORREY PINES RD, LA JOLLA, CA, 92037 (Reprint); SCRIPPS CLIN & RES FDN, RES INST, DEPT NEUROPHARMACOL, LA JOLLA, CA, 92037; SCRIPPS CLIN & RES FDN, RES INST, DEPT IMMUNOL, LA JOLLA, CA, 92037; SCRIPPS CLIN & RES FDN, RES INST, DEPT MOLEC BIOL, LA JOLLA, CA, 92037; YALE UNIV, SCH MED, BOYER CTR MOLEC MED, NEW HAVEN, CT, 06536. BIO-TECHNOLOGY (NOV 1995) Vol. 13, No. 11, pp. 1221-1224. ISSN: 0733-222X. Pub. country: USA. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Specific anti-tumor and anti-viral activities can be conferred on lymphocytic and myeloid effector cells by retargeting them with **bispecific** antibodies. These are antibodies which possess an anti-target binding region and a region capable of binding specific effector cell surface markers, For the rapid evaluation of recombinant human Fabs as **bispecific** antibodies, we have constructed a vector that allows for the conversion of Fabs into protein A fusion proteins, These can be used to generate **bispecific** antibodies when complexed to appropriate anti-effector cell immunoglobulins. As a model system, a protein A fusion derivative of a human recombinant anti-herpes simplex virus (HSV) Fab was constructed and complexed to OKT3, a T cell-activating antibody specific for CD3. This complex reduced HSV-2 yields in infected cells by about three logs relative to controls,when incubated on HSV-2-infected cell monolayers in the presence of IL-2-activated lymphocytes. The system described allows for the rapid evaluation of recombinant human Fabs as **bispecific** antibodies for therapeutic applications. In addition, Fab-protein A fusion proteins can be used in ELISA and other immune-assays with increased sensitivity.

L28 ANSWER 112 OF 128 MEDLINE
96032867 Document Number: 96032867. PubMed ID: 7565814. A genetically engineered **single-chain FV**/TNF molecule possesses the anti-tumor immunoreactivity of FV as well as the cytotoxic activity of tumor necrosis factor. Yang J; Moyana T; Xiang J. (Saskatoon Cancer Center, University of Saskatchewan, Canada.) MOLECULAR IMMUNOLOGY, (1995 Aug) 32 (12) 873-81. Journal code: 7905289. ISSN: 0161-5890. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Recombinant DNA techniques were used to clone, construct and express the fused gene FV-TNF in E. coli under control of the strong T7 bacteriophage promoter in the expression vector pT7-7-FV-TNF. The fusion protein FV/TNF in inclusion bodies from the bacteria homogenate was solubilized in the denaturing solution containing 6 mol/l guanidine and 0.3 mol/l DTT and refolded in refolding buffer containing 8 mmol/l GSSG. The FV/TNF was purified by ion exchange chromatography. The yield of FV/TNF was estimated at 10 mg/l. The purified FV/TNF displayed a single band of 42 kD under reducing conditions, whereas it showed three forms including its monomer (40/42 kD), its dimer (84 kD) and its trimer (126 kD) under non-reducing conditions. Our data showed that this fusion protein retained its bifunctional activities well, namely the anti-TAG72 immunoreactivity of the FV portion and the cytotoxic activity of the TNF moiety. Therefore, the FV/TNF fusion protein may prove useful in targeting the biological effect of TNF to tumor cells as well as in stimulating the immune destruction of tumor cells.

L28 ANSWER 113 OF 128 MEDLINE DUPLICATE 39
96129471 Document Number: 96129471. PubMed ID: 8581373. Expression of monovalent and bivalent antibody fragments in Escherichia coli. Grant S D; Cupit P M; Learmonth D; Byrne F R; Graham B M; Porter A J; Harris W J. (Department of Molecular and Cell Biology, University of Aberdeen, Marischal College, UK.) JOURNAL OF HEMATOTHERAPY, (1995 Oct) 4 (5) 383-8. Journal code: 9306048. ISSN: 1061-6128. Pub. country: United States. Language: English.

AB The technology of humanization of rodent antibodies has opened the way for a broad range of therapeutic antibodies with very low immunogenicity, which are, therefore, suitable for repeated dosing. Such intact antibodies have extended serum half-lives and biodistribution profiles very similar to human antibodies. For some applications, however, the ideal therapeutic should have reduced serum half-life and altered biodistribution patterns typical of antibody fragments, such as Fab or **single chain Fv**. **Bispecific** antibody fragments offer exciting additional therapeutic possibilities, but their successful manufacture and purification on a large scale require the development of new methods. Antibody fragments often assemble in Escherichia coli as monovalent fragments with reduced affinities. We describe the spontaneous assembly of bivalent antibody fragments in E. coli and methods of purification that yield either bivalent or monovalent molecules as required.

L28 ANSWER 114 OF 128 MEDLINE
1998298518 Document Number: 98298518. PubMed ID: 9634779. Calmodulin as a versatile tag for antibody fragments. Neri D; de Lalla C; Petrul H; Neri P; Winter G. (Cambridge Centre for Protein Engineering, MRC Centre, UK.) BIO/TECHNOLOGY, (1995 Apr) 13 (4) 373-7. Journal code: 8309273. ISSN: 0733-222X. Pub. country: United States. Language: English.

AB Calmodulin is a highly acidic protein (net charge -24 at pH 8.0 in the absence of calcium) that binds to peptide and organic ligands with high affinity (Ka > 10(9) M-1) in a calcium-dependent manner. We have exploited these properties to develop calmodulin as a versatile tag for antibody fragments. Fusions of calmodulin with **single chain Fv** fragments (scFv) could be expressed by secretion from bacteria in good yield (5-15 mg/l in shaker flasks), and purified from periplasmic lysates or broth to homogeneity in a single step, either by binding to anion-exchange resin (DEAE-Sephadex), or to an organic ligand of calmodulin (N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide-agarose). The antibody fusions could be detected by binding of fluorescently labeled peptide ligands, as illustrated by their use in confocal microscopy, fluorescent activated cell sorting and "band shift" gel electrophoresis. Moreover, the interaction between calmodulin and peptide ligands could provide a means of heterodimerization of proteins, as illustrated by the assembly of an antibody-calmodulin fusion with maltose binding protein tagged with a peptide ligand of calmodulin.

L28 ANSWER 115 OF 128 MEDLINE DUPLICATE 40
96092923 Document Number: 96092923. PubMed ID: 8521448. Redirection of cellular cytotoxicity. A two-step approach using recombinant **single-chain Fv** molecules. George A J; Titus J A; Jost C R; Kurucz I; Perez P; Andrew S M; Nicholls P J; Huston J S; Segal D M. (Department of Immunology, Royal Postgraduate Medical School, Hammersmith Hospital, London, UK.) CELL BIOPHYSICS, (1995 Jun) 26 (3) 153-65. Ref: 39. Journal code: 8002185. ISSN: 0163-4992. Pub. country: United States. Language: English.

AB In this article the authors discuss an indirect system for redirecting cellular cytotoxicity, which utilizes a "universal" **bispecific** antibody to redirect T-cells to kill cells targeted with **single-chain Fv** (sFv) fusion proteins that carry a peptide tag recognized by the **bispecific** antibody. This approach has a number of theoretical advantages in the immunotherapy of cancer.

L28 ANSWER 116 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
95:811838 The Genuine Article (R) Number: TF482. EXPRESSION IN ESCHERICHIA-COLI OF SOLUBLE AND M13 PHAGE-DISPLAYED FORMS OF A SINGLE-CHAIN ANTIBODY FRAGMENT SPECIFIC FOR DIGOXIN - ASSESSMENT IN A NOVEL DRUG IMMUNOASSAY. NAVARROTEULON I (Reprint); PERALDIROUX S; BERNARDI T; MARIN M; PIECHACZYK M; SHIRE D; PAU B; BIARDPIECHACZYK M. UFR PHARM, CNRS, UMR 9921, F-34060 MONTPELLIER 1, FRANCE; IGMM, CNRS, UMR 9942, MONTPELLIER, FRANCE; SANOFI RECH, LABEGE, FRANCE. IMMUNOTECHNOLOGY (MAY 1995) Vol. 1, No. 1, pp. 41-52. ISSN: 1380-2933. Pub. country: FRANCE . Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB A high affinity anti-digoxin **single-chain Fv** antibody fragment (scFv) was cloned from the mouse 2C2 hybridoma cell line and was functionally expressed both in the Escherichia coli periplasm as a soluble molecule and at the surface of the filamentous M13 bacteriophage as a fusion protein with the gene III minor coat protein. The 2C2 scFv sequence significantly differs from that of all the other anti-digoxin antibodies previously described. The 2C2 scFv shares with its parental monoclonal antibody a high specificity for digoxin, a cross-reactivity with active digoxin metabolites, but none with inactive metabolites, M13 phages displaying the 2C2 scFv at their surface have a high apparent affinity constant for digoxin (6.6 x 10(8) M(-1)) and were directly used to set up a novel type of immunoenzymatic assay for monitoring digoxin in sera of patients treated for either congestive heart failure or cardiac arrythmias. We thus report for the first time that phages displaying scFv may constitute a large source of important new reagents in the field of immunodiagnosis.

L28 ANSWER 117 OF 128 MEDLINE DUPLICATE 41
94165475 Document Number: 94165475. PubMed ID: 8120389. Redirection of T cell-mediated cytotoxicity by a recombinant **single-chain Fv** molecule. George A J; Titus J A; Jost C R; Kurucz I; Perez P; Andrew S M; Nicholls P J; Huston J S; Segal D M. (Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.) JOURNAL OF IMMUNOLOGY, (1994 Feb 15) 152 (4) 1802-11. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.
AB We have produced two **single-chain Fv** (sFv) proteins by bacterial periplasmic secretion, one sFv with specificity for the hapten DNP, and the other for the human transferrin receptor. After solubilization and refolding, we recovered several mg of active sFv per liter of bacterial culture. Each sFv bound to cells bearing the appropriate Ag and could be used to direct targeted cellular cytotoxicity. Targeting relied on a universal **bispecific** antibody designed to cross-link CD3 on the cytotoxic T cell with a peptide fused to the sFv carboxyl-terminus. The universal **bispecific** antibody was used in combination with the Ag-specific sFv to redirect human cytotoxic T cells to kill a variety of target cells. Such an approach has a number of advantages that may make it useful for the immunotherapy of cancer and other diseases.

L28 ANSWER 118 OF 128 MEDLINE DUPLICATE 42
95219383 Document Number: 95219383. PubMed ID: 7704531. Crystal structure of a diabody, a bivalent antibody fragment. Perisic O; Webb P A; Holliger P; Winter G; Williams R L. (Centre for Protein Engineering, MRC Centre, Cambridge, UK.) STRUCTURE, (1994 Dec 15) 2 (12) 1217-26. Journal code: 9418985. ISSN: 0969-2126. Pub. country: ENGLAND: United Kingdom. Language: English.
AB BACKGROUND: Diabodies are dimeric antibody fragments. In each polypeptide, a heavy-chain variable domain (VH) is linked to a light-chain variable domain (VL) but unlike **single-chain Fv** fragments, each antigen-binding site is formed by pairing of one VH and

one VL domain from the two different polypeptides. Diabodies thus have two antigen-binding sites, and can be **bispecific**. Direct structural evidence is lacking for the connections and dimeric interactions between the two polypeptides of the diabody. RESULTS: The 2.6 A resolution structure has been determined for a bivalent diabody with a flexible five-residue polypeptide linker between the (amino-terminal) VH and (carboxy-terminal) VL domains. The asymmetric unit of the crystal consists of four polypeptides comprising two diabodies; for one of these polypeptides the linker can be traced between the VH and VL domains. Within each diabody the two associated VH and VL domains make back-to-back interactions through the VH domains, and there is an extensive VL-VL interface between the two diabodies in the asymmetric unit. CONCLUSIONS: The structure of the diabody is very similar to that which had been predicted by molecular modelling. Diabodies directed against cell-surface antigens should be capable of bringing together two cells, such as in cell-targeted therapy, because the two antigen-binding sites of the diabody are at opposite ends of the molecule and separated by approximately 65 A.

L28 ANSWER 119 OF 128 MEDLINE DUPLICATE 43
95021313 Document Number: 95021313. PubMed ID: 7935496. Recombinant **single-chain Fv** fragments carrying C-terminal cysteine residues: production of bivalent and biotinylated miniantibodies. Kipriyanov S M; Dubel S; Breitling F; Kontermann R E; Little M. (Recombinant Antibody Research Group (FSP 4/0445), German Cancer Research Center, Heidelberg.) MOLECULAR IMMUNOLOGY, (1994 Oct) 31 (14) 1047-58. Journal code: 7905289. ISSN: 0161-5890. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A murine antibody **single-chain Fv** (scFv) fragment carrying five C-terminal histidine residues preceded by a cysteine residue and a marker peptide was expressed in Escherichia coli. Its variable heavy (VH) and light (VL) domains are derived from the mouse monoclonal antibody mAb215, which is specific for the largest subunit of RNA polymerase II of Drosophila melanogaster. ScFv' monomers, covalently linked (scFv')2 and non-covalent dimers, as well as aggregated antibody fragments, were isolated from an E. coli cell paste by immobilized metal affinity chromatography in 6 M urea followed by a renaturation procedure that does not use any sulfhydryl agents. In a final step, the components were separated by size exclusion chromatography. All the recombinant antibody fractions demonstrated high antigen-binding activity and specificity as shown by ELISA and Western blot analysis. Affinity measurements carried out by competitive immunoassays showed that covalently linked (scFv')2 have binding constants quite close to those of the parental monoclonal antibodies and four-fold higher than scFv' monomers. ScFv derivatives, specifically biotinylated through the free sulfhydryl group, recognize the corresponding antigen in ELISA and Western blot analysis, thus demonstrating the possibility of using chemically modified scFv antibodies for immunodetection.

L28 ANSWER 120 OF 128 MEDLINE DUPLICATE 44
95107942 Document Number: 95107942. PubMed ID: 7809028. Multivalent Fvs: characterization of **single-chain Fv** oligomers and preparation of a **bispecific** Fv. Whitlow M; Filpula D; Rollence M L; Feng S L; Wood J F. (Research and Development Department, Enzon, Incorporated, Piscataway, NJ 08854-3998.) PROTEIN ENGINEERING, (1994 Aug) 7 (8) 1017-26. Journal code: 8801484. ISSN: 0269-2139. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **Single-chain Fv** proteins are known to aggregate and form multimeric species. We report here that these molecules represent a new class of molecular assembly, which we have termed multivalent Fvs. Each binding site in a multivalent Fv comprises the variable light-chain (VL) domain from a **single-chain Fv**, and the variable heavy-chain (VH) domain from a second

single-chain Fv. Each **single-chain Fv** in a multivalent Fv is part of two binding sites. We have characterized the multivalent forms of the 4-4-20, CC49 and B6.2 sFvs. The degree of multivalent Fv formation is linker-dependent. Multivalent Fvs cannot form in the absence of an intact linker. Multivalent Fvs can be stabilized by their antigen. The conversion between different forms of the multivalent Fvs can be catalyzed by disassociating agents such as 0.5 M guanidine hydrochloride with 20% ethanol. Multivalent Fvs have significantly different stabilities depending on the specific variable domains from which they are constructed. Two models have been proposed for the structure of a multivalent Fv. We have tested each model by attempting to produce a heterodimer from the anti-fluorescein 4-4-20 and anti-tumor CC49 variable regions. We successfully produced a 4-4-20/CC49 heterodimer that comprises two mixed sFvs. The first mixed sFv is composed of the 4-4-20 VL domain, a 12 residue linker and the CC49 Vh domain. The second mixed sFv is composed of a CC49 VL domain, a 12 residue linker and the 4-4-20 VH domain. The 4-4-20/CC49 heterodimer bound both fluorescein and the tumor-associated glycoprotein-72 antigen. These results support a VH/VL 'rearrangement' model in which each variable domain of a multivalent Fv binding site comes from a different polypeptide chain.

L28 ANSWER 121 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1994:147608 Document No.: PREV199497160608. Chimeric **bispecific** antibody binding sites (times BABS): Design of a second combining site within the Fv region and recovery of **single-chain Fv** binding activity after integration of new binding loops between beta-strands of V domains. Keck, P. C.; Tai, M.-S.; Oppermann, H.; Huston, J. S.. Creative Biomolecules Inc., 45 South Street, Hopkinton, MA 01748 USA. Biophysical Journal, (1994) Vol. 66, No. 2 PART 2, pp. A341. Meeting Info.: Thirty-eighth Annual Meeting of the Biophysical Society New Orleans, Louisiana, USA March 6-10, 1994 ISSN: 0006-3495. Language: English.

L28 ANSWER 122 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)
94:82552 The Genuine Article (R) Number: MU462. CHIMERIC **BISPECIFIC** ANTIBODY-BINDING SITES (CHI-BABS) - DESIGN OF A 2ND COMBINING SITE WITHIN THE FV REGION AND RECOVERY OF **SINGLE-CHAIN FV** BINDING-ACTIVITY AFTER INTEGRATION OF NEW BINDING LOOPS BETWEEN BETA-STRANDS OF V-DOMAINS. KECK P C (Reprint); TAI M S; OPPERMANN H; HUSTON J S. CREAT BIOMOLECULES INC, HOPKINTON, MA, 01748. BIOPHYSICAL JOURNAL (FEB 1994) Vol. 66, No. 2, Part 2, pp. A341. ISSN: 0006-3495. Pub. country: USA. Language: ENGLISH.

L28 ANSWER 123 OF 128 CAPLUS COPYRIGHT 2002 ACS
1993:647978 Document No. 119:247978 Monomeric and dimeric antibody-fragment fusion proteins and their manufacture with recombinant cells. Plueckthun, Andreas; Pack, Peter (Merck Patent G.m.b.H., Germany). PCT Int. Appl. WO 9315210 A1 19930805, 44 pp. DESIGNATED STATES: RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1993-EP82 19930115. PRIORITY: EP 1992-101069 19920123.

AB **Single-chain Fv** fragments fused (optionally through a linker peptide) to a peptide which can dimerize with another peptide by noncovalent interactions, and dimeric fusion proteins formed by this interaction are described. These monomeric and dimeric fusion proteins can be prepd. by expression of chimeric genes in a suitable host cell, e.g. E. coli. Thus, a vector contg. genes for an scFv-linker-fos protein leucine zipper peptide and an scFv-linker-jun protein leucine zipper expressed from a single promoter was prepd. E. coli contg. this vector produced a **bispecific** miniantibody which was purified by affinity chromatog. and which was characterized with respect to specificity and affinity of ligand binding.

L28 ANSWER 124 OF 128 MEDLINE DUPLICATE 45
93283044 Document Number: 93283044. PubMed ID: 8507403. Bacterial expression of immunoglobulin fragments. Skerra A. (Max-Planck-Institut fur Biophysik, Frankfurt am Main, Germany.) CURRENT OPINION IN IMMUNOLOGY, (1993 Apr) 5 (2) 256-62. Ref: 68. Journal code: 8900118. ISSN: 0952-7915. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The expression of Ig fragments in Escherichia coli permits rapid access to engineered molecules with antigen-binding properties. While the expression in a functional state by secretion to the periplasm is the standard method for the production of Fv and Fab fragments, **single chain Fv** fragments are mainly produced by refolding from insoluble aggregates. Although all of these Ig fragments serve as valuable aids in the study of antigen binding, their different biochemical properties must be considered when using them as research tools or for medical applications. In addition to these simple univalent antibody fragments, the bacterial expression of bivalent and **bispecific** versions and of hybrid proteins with novel effector functions is gaining increasing importance.

L28 ANSWER 125 OF 128 CAPLUS COPYRIGHT 2002 ACS
1993:58113 Document No. 118:58113 Tetravalent **bispecific** receptors derived from immunoglobulins, their preparation and use. Bosslet, Klaus; Seemann, Gerhard (Behringwerke A.-G., Germany). Eur. Pat. Appl. EP 517024 A2 19921209, 35 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, PT, SE. (German). CODEN: EPXXDW. APPLICATION: EP 1992-108381 19920518. PRIORITY: DE 1991-4118120 19910603.

AB Tetravalent **bispecific** receptors consisting of heterologous F(ab')2 fragments with the CH1 domains substituted by CH3 domains or by a **single chain Fv** fragment are manufd. by expression of the corresponding genes in animal cell culture. These Ig derivs. are useful in the treatment of malignant tumors (no data). The genes were constructed by std. methods and introduced into animal cells and transfected clones synthesizing the receptor were identified.

L28 ANSWER 126 OF 128 MEDLINE DUPLICATE 46
92144568 Document Number: 92144568. PubMed ID: 1737014. Miniantibodies: use of amphipathic helices to produce functional, flexibly linked dimeric FV fragments with high avidity in Escherichia coli. Pack P; Pluckthun A. (Genzentrum Universitat Munchen, Max-Planck-Institut fur Biochemie, Martinsried, FRG.) BIOCHEMISTRY, (1992 Feb 18) 31 (6) 1579-84. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB We have designed dimeric antibody fragments that assemble in Escherichia coli. They are based on **single-chain FV** fragments, with a flexible hinge region from mouse IgG3 and an amphiphilic helix fused to the C-terminus of the antibody fragment. The sequence of the helix was taken either from that of a previously reported four-helix bundle design or from a leucine zipper, optionally extended with a short cysteine-containing peptide. The bivalent fragments associate in vivo, either with covalent linkage or with a monomer-dimer equilibrium, and results from ultracentrifugation sedimentation studies and SDS-PAGE are consistent with dimers. All constructs are able to bind to surface-bound antigen under conditions in which only bivalent but not monovalent antibody fragments bind. The covalent bundle helix construct shows binding characteristics nearly identical to those of the much larger whole mouse antibody, resulting in substantially more stable immunoglobulin-antigen complexes than in the case of monovalent fragments. This modular design of natural and engineered protein domains directly leads to a boost of avidity, and it allows the construction of **bispecific** antibody fragments in functional form in E. coli.

L28 ANSWER 127 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1991:239075 Document No.: BR40:113240. PRODUCTION OF A **BISPECIFIC**

ANTIBODY BY LINKAGE OF TWO RECOMBINANT **SINGLE CHAIN** **FV** MOLECULES. GEORGE A J T; ANDREW S M; PEREZ P; NICHOLLS P J; HUSTON J S; SEGAL D M. EXPERIMENTAL IMMUNOLOGY BRANCH, NCI, BETHESDA, MD. 20892.. MEETING ON MONOCLONAL ANTIBODIES HELD AT THE 20TH ANNUAL MEETING OF THE KEYSTONE SYMPOSIA ON MOLECULAR AND CELLULAR BIOLOGY, DENVER, COLORADO, USA, MARCH 10-16, 1991. J CELL BIOCHEM SUPPL. (1991) 15 (PART E), 127. CODEN: JCBSD7. Language: English.

L28 ANSWER 128 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1997:296286 Document No.: PREV199799595489. Design and expression of a stable **bispecific** scFv dimer with affinity for both glycophorin and N9 neuraminidase. Atwell, John L. (1); Pearce, Lesley A.; Lah, Maria; Gruen, L. Clem; Kortt, Alexander A.; Hudson, Peter J.. (1) CSIRO, Div. Biomolecular Eng., 343 Royal Parade, Parkville, 3052 VIC Australia. Molecular Immunology, Vol. 33, No. 17-18, pp. 1301-1312. ISSN: 0161-5890. Language: English.

AB We have designed and produced a stable **bispecific** scFv dimer (bisFv) by non-covalent association of two hybrid V-H-V-L pairs derived from an anti-neuraminidase antibody (NC10) and an anti-glycophorin antibody (1C3). The bisFv dimer was demonstrated to have binding activity to the two respective target antigens and was evaluated as a reagent for rapid whole blood agglutination assays. The bisFv was expressed in the periplasm of Escherichia coli, from a secretion vector which comprised two cistrons in tandem under the control of a single lac promoter, inducible with IPTG. Each cistron encoded one of the hybrid V-H-V-L pairs, with V domains separated by a linker region encoding the five amino acids, Gly-4Ser. The short linker region was designed to prevent association of V-H and V-L regions of the same molecule and favour the formation of dimers. The protein synthesized from each hybrid scFv cistron was directed to the E. coli periplasm by the inclusion of distinctive signal secretion sequences preceding each hybrid gene; from pel B of Erwinia cartovora and from gene III of fd phage. The bisFv was affinity-purified from culture supernatants via the C-terminal tag epitope FLAG and was shown, by FPLC on a Superose 6 column, to be consistent in size with that of a scFv dimer. The bisFv was stable for more than 4 months at 4 degree C and was shown by BIAcore analysis to bind to either target antigen, human glycophorin, or tern N9 neuraminidase. Simultaneous binding to both target antigens was demonstrated when a pre-formed bisFv-neuraminidase complex was shown to bind to immobilized glycophorin. In whole blood agglutination assays, the bisFv dimer was able to agglutinate red blood cells when crosslinked with an anti-idiotype antibody (3-2G12) binding to the NC10 combining site, but no agglutination occurred on binding the antigen neuraminidase. These results are a function of the topology of the epitopes on neuraminidase and have implications for the use of relatively rigid bifunctional molecules (as bisFv dimers) to cross link two large membrane-anchored moieties, in this case, red blood cell glycophorin and neuraminidase, an M-r 190 000 tetramer.

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=> s (zhu z?/au)
L31       9951 (ZHU Z?/AU)

=> s l31 and bispecific
L32         50 L31 AND BISPECIFIC

=> dup remove l32
PROCESSING COMPLETED FOR L32
L33          16 DUP REMOVE L32 (34 DUPLICATES REMOVED)

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L33  ANSWER 1 OF 16     MEDLINE                          DUPLICATE 1
2002411062  Document Number: 22155357.     PubMed ID: 12165442.    Fab-scFv
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fusion protein: an efficient approach to production of **bispecific** antibody fragments. Lu Dan; Jimenez Xenia; Zhang Haifan; Bohlen Peter; Witte Larry; **Zhu Zhenping**. (Department of Antibody Technology, ImClone Systems Incorporated, 180 Varick Street, New York, NY 10014, USA.) JOURNAL OF IMMUNOLOGICAL METHODS, (2002 Sep 15) 267 (2) 213-26. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB The clinical development of **bispecific** antibodies (BsAb) as therapeutics has been hampered by the difficulty in preparing the materials in sufficient quantity and quality by traditional methods. Here, we describe an efficient approach for the production of a novel **bispecific** antibody fragment by genetically fusing a single-chain Fv (scFv) to the C-terminus of either the light chain or the heavy chain of a Fab fragment of different antigen-binding specificity. The **bispecific** Fab-scFv fragments were expressed in a single Escherichia coli host and purified to homogeneity by a one-step affinity chromatography. Two different versions of the **bispecific** Fab-scFv fragments were constructed using two antibodies directed against the two tyrosine kinase receptors of vascular endothelial growth factor. These **bispecific** antibody fragments not only retained the antigen-binding capacity of each of the parent antibodies, but also are capable of binding to both targets simultaneously as demonstrated by a cross-linking ELISA. Further, the **bispecific** antibodies were comparable to their parent antibodies in their potency in blocking ligand binding to the receptors and in inhibiting ligand-induced biological activities. This design for BsAb fragments should be applicable to any pair of antigen specificities.

L33 ANSWER 2 OF 16 MEDLINE DUPLICATE 2

2002082965 Document Number: 21667984. PubMed ID: 11809528. Efficient inhibition of human B-cell lymphoma xenografts with an anti-CD20 x anti-CD3 **bispecific** diabody. Xiong Dongsheng; Xu Yuanfu; Liu Hanzhi; Peng Hui; Shao Xiaofeng; Lai Zenzu; Fan Dongmei; Yang Min; Han Junling; Xie Yong; Yang Chunzheng; **Zhu Zhenping**. (State Key Laboratory of Experimental Hematology, Institute of Hematology, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin 300020, People's Republic of China.) CANCER LETTERS, (2002 Mar 8) 177 (1) 29-39. Journal code: 7600053. ISSN: 0304-3835. Pub. country: Ireland. Language: English.

AB **Bispecific** antibodies have been exploited both as cancer immunodiagnostics and as cancer therapeutics, and have shown promise in several clinical trials in cancer imaging and therapy. A number of **bispecific** antibodies against B-cell markers have been shown to be effective in vitro in mediating tumor cell lysis and in vivo in inhibiting tumor growth in animal models. We have constructed a **bispecific** diabody from the variable genes encoding two hybridoma-derived monoclonal antibodies directed against human CD20 on B cells and CD3 on T cells. The anti-CD20 x anti-CD3 diabody was expressed in a single Escherichia coli host and purified by a one-step affinity chromatography. The **bispecific** diabody bound as efficiently to both CD20- and CD3-positive cells as the respective parental antibodies, and was capable of cross-linking CD20-positive tumor cells and human T lymphocytes as shown by cellular rosetting. The diabody effectively lysed human B-lymphoma cells in the presence of T-enriched human peripheral blood lymphocytes (PBL). Further, when combined with human PBL and interleukin-2, the diabody significantly prolonged the survival of nude mice inoculated with human B-lymphoma cells. Taken together, our results suggest that an anti-CD20 x anti-CD3 diabody may have significant clinical application in the treatment of human CD20-positive B-cell malignancies.

L33 ANSWER 3 OF 16 CAPLUS COPYRIGHT 2002 ACS

2001:868530 Document No. 136:19113 **Bispecific** immunoglobulin-like antigen binding proteins and method of production. **Zhu, Zhenping**

(Imclone Systems Incorporated, USA). PCT Int. Appl. WO 2001090192 A2 20011129, 64 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US16924 20010524. PRIORITY: US 2000-PV206749 20000524.

AB The present invention is directed to **bispecific** antigen-binding protein. These **bispecific** antigen-binding proteins are optimized in their avidity for antigen(s) but maintain their ability to function as a natural antibody, including the ability to activate complement mediated cytotoxicity and antibody dependent cellular toxicity. Natural IgG Igs are monospecific and bivalent, having two binding domains which are specific for the same epitope. By contrast, an IgG type antigen-binding protein of the invention is **bispecific** and bivalent, having a binding domain on each light chain for one epitope and a binding domain on each heavy chain specific for a second epitope. The design of the present antigen-binding proteins provides for efficient prodn. such that substantially all of the antigen-binding proteins produced are assembled in the desired configuration.

L33 ANSWER 4 OF 16 MEDLINE

2001538782 Document Number: 21469635. PubMed ID: 11585724. Complete inhibition of vascular endothelial growth factor (VEGF) activities with a bifunctional diabody directed against both VEGF kinase receptors, fms-like tyrosine kinase receptor and kinase insert domain-containing receptor. Lu D; Jimenez X; Zhang H; Wu Y; Bohlen P; Witte L; **Zhu Z**. (Department of Molecular and Cell Biology, ImClone Systems Inc., New York, New York 10014, USA.) CANCER RESEARCH, (2001 Oct 1) 61 (19) 7002-8. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Vascular endothelial growth factor (VEGF) binds to and mediates its activity mainly through two tyrosine kinase receptors, VEGF receptor 1 [or fms-like tyrosine kinase receptor (Flt-1)] and VEGF receptor 2 [or kinase insert domain-containing receptor (KDR)]. Numerous studies have shown that overexpression of VEGF and its receptor plays an important role in tumor-associated angiogenesis and hence in both tumor growth and metastasis. We demonstrated previously that antagonistic antibodies to KDR specifically inhibited VEGF-stimulated receptor activation, cell migration, and endothelial cell mitogenesis. Here we constructed a recombinant bifunctional diabody that is capable of blocking both Flt-1 and KDR from binding to their ligands, including VEGF and placenta growth factor (PlGF). The diabody was expressed in Escherichia coli and purified by single-step affinity chromatography. The diabody retained the capacity to bind both KDR and Flt-1 and effectively blocked interaction between KDR and VEGF, Flt-1 and VEGF, and Flt-1 and PlGF. Furthermore, the diabody is a stronger inhibitor than its parent antibodies to VEGF-stimulated mitogenesis of human endothelial cells, as well as both VEGF- and PlGF-induced migration of human leukemia cells. Taken together, our results suggest that dual receptor blockade with the bifunctional diabody may prove to be a more efficient approach in inhibiting VEGF-stimulated angiogenesis.

L33 ANSWER 5 OF 16 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 3

2001:353815 The Genuine Article (R) Number: 424RE. **Bispecific** antibody and its clinical applications in cancer. Xu Y F (Reprint); Yang C Z; **Zhu Z P**. Chinese Acad Med Sci, Peking Union Med Coll, Inst Hematol, Natl Lab Expt Hematol, Tianjin 300020, Peoples R China (Reprint). CHINESE SCIENCE BULLETIN (MAR 2001) Vol. 46, No. 5, pp. 353-358.

Publisher: SCIENCE PRESS. 16 DONGHUANGCHENGGEN NORTH ST, BEIJING 100717, PEOPLES R CHINA. ISSN: 1001-6538. Pub. country: Peoples R China. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Bispecific** antibody (BsAb) usually consists of two different antigen-binding arms, by which it is capable of simultaneously binding to target cells and effector cells, and can directly mediate the killing of target cells by retargeting and activating effector cells. The development of BsAb research goes through three main stages: chemical crosslinking of murine-derived monoclonal antibody, hybrid hybridomas and engineered BsAb. Among them, engineered BsAb has more formats than the other two, such as diabody, ScdHLX, ScZip, ScCH3, ScFab and BsIgG, etc. Compared with former murine-derived BsAbs, engineered BsAb has lower immunogenicity and stronger penetrating capacity, and currently, some of them appear suitable for clinical application in yields and qualities. Up to now, several phase I and phase II clinical studies of BsAb, for instance, some (Fab')(2) and Diabodies, have been performed. Among those BsAbs, anti-CD3/anti-tumor BsAbs is most common, they not only can activate T cell and induce CD3AK cytotoxic activity in in vitro experiment, and inhibit the growth of tumor on tumor-bearing mouse by retargeting T cells to lyse tumor cells, but also offer great promise in the therapy of some malignancies in clinic, especially of some advanced cancers as well as elimination of minimal residual tumors, indicated by increasing the tumor/blood ratio of antibody in patients and improving the natural killer cell (NK) anti-tumor activity in tumor sites, and also presenting of an increase level in TNF-alpha, INF-gamma, IL-6, IL-8, IL-10 and soluble CD25, etc. The responses are also shown via improving the quality of life and prolonging the survival of partial patients. The "Knobs into Holes" technology is a new strategy emerging during research on engineered BsAb, it is likely to be useful for heterodimerization and can improve the quantity, purity and stability of BsAb, it is also anticipated to increase the clinical potential of BsAb in the future.

L33 ANSWER 6 OF 16 CAPLUS COPYRIGHT 2002 ACS
2001:625400 Document No. 136:323667 Study on construction and expression of anti-CD3/anti-CD20 diabody. Xiong, Dongsheng; Xu, Yuanfu; Yang, Chunzheng; Peng, Hui; Shao, Xiaofeng; Lai, Zengzu; Fan, Dongmei; Yang, Ming; **Zhu, Zhenping** (The State Key Laboratory of Experimental Hematology, Institute of Hematology, CAMS & PUMC, Tianjin, 300020, Peop. Rep. China). Zhongguo Mianyixue Zazhi, 17(7), 339-342, 347 (Chinese) 2001. CODEN: ZMZAEE. ISSN: 1000-484X. Publisher: Zhongguo Mianyixue Zazhi Bianjibu.

AB The construction and expression of anti-CD3/anti-CD20 Diabody and identification of its biol. were studied. PCR and overlap PCR were used to construct anti-CD3/anti-CD20 Diabody. DNA sequence was analyzed by the Terminus of Dideoxy Nucleotide. The product was purified by affinity chromatog. and analyzed by both the detection of Western blot and size exclusion chromatog.; its antigen-binding activity was examd. by FACS and rosetting assay. The data of DNA sequence showed that the anti-CD3/anti-CD20 Diabody was correct. The diabody was recovered in high yield (up to 1 mg/mL) after E-taq purifn. and predominantly (90%) as a dimer. The Diabody binded Jurkat cells (CD3+) and Daudi cells, resp. Furthermore, the Diabody was capable of simultaneous binding to Jurkat cells and Daudi cells as shown by cellular rosetting. The anti-CD3/anti-CD20 BsF(ab')2 was first recast into the Diabody format and succeeded to obtain high level expression. The results of biol. activity expts. indicated that the Diabody could bind to Jurkat cells and Daudi cells.

L33 ANSWER 7 OF 16 CAPLUS COPYRIGHT 2002 ACS
2001:368463 Document No. 136:100058 Development of human lymphoma nude mouse xenograft model and anti-CD20/CD3 **bispecific** antibody therapy of human B cell tumor. Shao, Xiaofeng; Yang, Chunzheng; Xiong, Dongsheng;

Xu, Yuanfu; Peng, Hui; Lai, Zengzu; **Zhu, Zhenping** (Institute of Hematology, Chinese Academy of Medical Sciences&+ PUMC, Tianjin, 300020, Peop. Rep. China). Zhongguo Zhongliu Linchang, 28(3), 217-219 (Chinese) 2001. CODEN: ZZLIEP. ISSN: 1000-8179. Publisher: Zhongguo Zhongliu Linchang Bianji Weiyuanhui.

AB The human Raji tumor cell nude mouse xenograft model was developed for study of antitumor effect of anti-CD20/CD3 **bispecific** antibody. Human Raji tumor cells (about 1X107) were inoculated into abdominal cavity of nude mouse, which 0.2ml pristane have been given two times, 3 days after irradiated with 3-Gy dose. Antibody therapy began in the next day. Injection of human T cell and anti-CD20/CD3 which have been activated in advance was given i.p. once/w for 4 times. Tumor grew in all 38 nude mice with a incidence rate of 100%. The tumor cells mainly distributed in abdominal cavity, mesentery and lymph nodes nearby the intestinal wall. In treatment group, the pos. rate of CD19, CD20 and HLA-DR was more than 95% and all the animals were alive more than 100 days, while in control group, all animals were alive only 40 days. Human B cell tumor nude mouse xenograft model was successfully developed and the result showed that anti-CD20/CD3 **bispecific** antibody displays good antitumor effect in nude mouse xenograft model.

L33 ANSWER 8 OF 16 MEDLINE DUPLICATE 4

2000295268 Document Number: 20295268. PubMed ID: 10835110. An efficient route to the production of an IgG-like **bispecific** antibody. Zuo Z; Jimenez X; Witte L; **Zhu Z**. (Department of Molecular and Cell Biology, ImClone Systems Incorporated, 180 Varick Street, New York, NY 10014, USA.) PROTEIN ENGINEERING, (2000 May) 13 (5) 361-7. Journal code: 8801484. ISSN: 0269-2139. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Production of IgG-form **bispecific** antibody (BsAb-IgG) by co-expressing two antibodies in transfected cells is often inefficient owing to the unwanted pairing between the component heavy and light chains. We have developed an efficient method for the production of a novel IgG-like BsAb by using the natural dimerization mechanism between IgG heavy and light chains. Two single-chain Fv (scFv) of different specificity are fused to the constant domain of human kappa chain (C(L)) and the first constant domain of human heavy chain (C(H1)), to form two polypeptides, (scFv)(1)-C(L) and (scFv)(2)-C(H1)-C(H2)-C(H3), respectively. Co-expression of the two polypeptides in mammalian cells results in the formation of a covalently linked IgG-like hetero-tetramer, Bs(scFv)(4)-IgG, with dual specificity. Our approach yields a homogeneous **bispecific** IgG-like antibody product with each molecule containing four antigen binding sites, two for each of its target antigens. A Bs(scFv)(4)-IgG was prepared using two scFv antibodies each directed against a different epitope of a vascular endothelial growth factor receptor, the kinase insert domain-containing receptor (KDR). The Bs(scFv)(4)-IgG is capable of simultaneously binding to the two epitopes on the receptor. Further, the Bs(scFv)(4)-IgG also retains the antigen-binding efficacy and biological activity of its component antibodies.

L33 ANSWER 9 OF 16 MEDLINE DUPLICATE 5

2000062952 Document Number: 20062952. PubMed ID: 10594363. Acquired antagonistic activity of a **bispecific** diabody directed against two different epitopes on vascular endothelial growth factor receptor 2. Lu D; Kotanides H; Jimenez X; Zhou Q; Persaud K; Bohlen P; Witte L; **Zhu Z**. (Department of Molecular and Cell Biology, ImClone Systems, 180 Varick Street, New York, NY 10014, USA.) JOURNAL OF IMMUNOLOGICAL METHODS, (1999 Nov 19) 230 (1-2) 159-71. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB **Bispecific** antibody (BsAb) technology has been successfully used as a means to construct novel antibody (Ab) molecules with increased avidity for binding, by combining two Ab or their fragments directed

against different epitopes within the same antigen. Using two single chain antibodies (scFv) isolated from a phage display library, we have constructed a **bispecific** diabody directed against two different epitopes on the extracellular domain (ECD) of human vascular endothelial growth factor receptor 2 (VEGFR2), the kinase-insert domain-containing receptor (KDR). Neither of the parent scFv blocks KDR/VEGF interactions or inhibits VEGF-induced receptor activation. The diabody binds to KDR with an affinity that is 1.5- to 3-fold higher than its parent scFv, mainly due to a much slower dissociation rate (k(off)), which is approximately 17- to 26-fold slower than that of the individual scFv. In addition, the diabody binds simultaneously to, and thus cross-links, the two epitopes on the receptor(s). It is rather unexpected that the diabody effectively blocked KDR/VEGF interactions, and inhibited both VEGF-induced activation of the receptor and mitogenesis of human endothelial cells. Taken together, our results suggest that the diabody is most likely to exert its effect through steric hindrance and/or causing major conformational changes of the receptor. This is the first report on the construction of a **bispecific** diabody with acquired novel antagonistic activity.

L33 ANSWER 10 OF 16 MEDLINE DUPLICATE 6

1998325681 Document Number: 98325681. PubMed ID: 9661204. An efficient route to human **bispecific** IgG. Merchant A M; **Zhu Z**; Yuan J Q; Goddard A; Adams C W; Presta L G; Carter P. (Department of Molecular Oncology, Genentech Inc., South San Francisco, CA 94080, USA.) NATURE BIOTECHNOLOGY, (1998 Jul) 16 (7) 677-81. Journal code: 9604648. ISSN: 1087-0156. Pub. country: United States. Language: English.

AB Production of **bispecific** IgG (BsIgG) by coexpressing two different antibodies is inefficient due to unwanted pairings of the component heavy and light chains. To overcome this problem, heavy chains were remodeled for heterodimerization using engineered disulfide bonds in combination with previously identified "knobs-into-holes" mutations. One of the variants, S354C:T366W/Y349'C:T366'S:L368'A:Y407++ +'V, gave near quantitative (approximately 95%) heterodimerization. Light chain mispairing was circumvented by using an identical light chain for each arm of the BsIgG. Antibodies with identical light chains that bind to different antigens were identified from an scFv phage library with a very restricted light chain repertoire for the majority (50/55) of antigen pairs tested. A BsIgG capable of simultaneously binding to the human receptors HER3 and cMpI was prepared by coexpressing the common light chain and corresponding remodeled heavy chains followed by protein A chromatography. The engineered heavy chains retain their ability to support antibody-dependent cell-mediated cytotoxicity as demonstrated with an anti-HER2 antibody.

L33 ANSWER 11 OF 16 MEDLINE DUPLICATE 7

97253444 Document Number: 97253444. PubMed ID: 9098887. Remodeling domain interfaces to enhance heterodimer formation. **Zhu Z**; Presta L G; Zapata G; Carter P. (Department of Molecular Oncology, Genentech Inc., South San Francisco, California 94080, USA.) PROTEIN SCIENCE, (1997 Apr) 6 (4) 781-8. Journal code: 9211750. ISSN: 0961-8368. Pub. country: United States. Language: English.

AB An anti-p185HER2/anti-CD3 humanized **bispecific** diabody was previously constructed from two cross-over single-chain Fv in which YH and VL domains of the parent antibodies are present on different polypeptides. Here this diabody is used to evaluate domain interface engineering strategies for enhancing the formation of functional heterodimers over inactive homodimers. A disulfide-stabilized diabody was obtained by introducing two cysteine mutations, VL L46C and VH D101C, at the anti-p185HER2.VL/VH interface. The fraction of recovered diabody that was functional following expression in Escherichia coli was improved for the disulfide-stabilized compared to the parent diabody (> 96% versus 72%), whereas the overall yield was > 60-fold lower. Eleven "knob-into-hole" diabodies were designed by molecular modeling of sterically complementary

mutations at the two VL/VH interfaces. Replacements at either interface are sufficient to improve the fraction of functional heterodimer, while maintaining overall recoverable yields and affinity for both antigens close to that of the parent diabody. For example, diabody variant v5 containing the mutations VL Y87A:F98M and VH V37F:L45W at the anti-p185HER2 VL/VH interface was recovered as 92% functional heterodimer while maintaining overall recovered yield within twofold of the parent diabody. The binding affinity of v5 for p185HER2 extracellular domain and T cells is eightfold weaker and twofold stronger than for the parent diabody, respectively. Domain interface remodeling based upon either sterically complementary mutations or interchain disulfide bonds can facilitate the production of a functional diabody heterodimer. This study expands the scope of domain interface engineering by demonstrating the enhanced assembly of proteins interacting via two domain interfaces.

L33 ANSWER 12 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1996:257293 Document No.: PREV199698813422. Engineering and production of humanized anti-p185-HER2/anti-CD3 **bispecific** diabodies for efficient tumor cell lysis. **Zhu, Zhenping;** Zapata, Gerardo; Shalaby, Refaat; Snedecor, Brad; Chen, Han; Carter, Paul. Genentech Inc., South San Francisco, CA 94080 USA. Proceedings of the American Association for Cancer Research Annual Meeting, (1996) Vol. 37, No. 0, pp. 468. Meeting Info.: 87th Annual Meeting of the American Association for Cancer Research Washington, D.C., USA April 20-24, 1996 ISSN: 0197-016X. Language: English.

L33 ANSWER 13 OF 16 MEDLINE DUPLICATE 8
1998299952 Document Number: 98299952. PubMed ID: 9636323. High level secretion of a humanized **bispecific** diabody from Escherichia coli. **Zhu Z;** Zapata G; Shalaby R; Snedecor B; Chen H; Carter P. (Department of Molecular Oncology, Genentech Inc., South San Francisco, CA 94080, USA.) BIO/TECHNOLOGY, (1996 Feb) 14 (2) 192-6. Journal code: 8309273. ISSN: 0733-222X. Pub. country: United States. Language: English.
AB Clinical development of **bispecific** antibodies (BsAb) has been effectively stymied by the lack of efficient production methods. We therefore attempted to produce a humanized BsAb fragment using an expression system that has proved very successful for secretion of monospecific Ab fragments from E. coli. An anti-p185HER2/anti-CD3 BsF(ab')2 was first recast into the diabody format and then periplasmically secreted from E. coli grown to high cell density in a fermentor. The diabody was recovered in very high yield (up to 935 mg/l) after protein A purification and predominantly (> or = 80%) as a dimer as judged by size exclusion chromatography. Diabody dimers were found to be mainly functional heterodimers (approximately 75%) by titration with p185HER2 extracellular domain. The diabody binds p185HER2 extracellular domain and human T lymphocytes with affinities close to those of the parent BsF(ab')2. Furthermore, the diabody is capable of simultaneous binding to tumor cells overexpressing p185HER2 and CD3 on T cells as shown by cellular rosetting. The diabody is equally potent as the parent BsF(ab')2 in retargeting IL-2 activated T-enriched peripheral blood lymphocytes to lyse tumor cells overexpressing p185HER2.

L33 ANSWER 14 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1995:187063 Document No.: PREV199598201363. Engineering high affinity humanized anti-p185-HER2 x anti-CD3 **bispecific** F(ab')-2 for efficient lysis of p185-HER2 over-expressing tumor cells. **Zhu, Zhenping (1);** Phillips, Gail; Carter, Paul (1). (1) Dep. Cell Genetics, Genentech Inc., 460 Point San Bruno Blvd., South San Francisco, CA 94080 USA. Proceedings of the American Association for Cancer Research Annual Meeting, (1995) Vol. 36, No. 0, pp. 484. Meeting Info.: Eighty-sixth Annual Meeting of the American Association for Cancer Research Toronto, Ontario, Canada March 18-22, 1995 ISSN: 0197-016X. Language: English.

L33 ANSWER 15 OF 16 MEDLINE DUPLICATE 9
96129484 Document Number: 96129484. PubMed ID: 8581386. Toward the production of **bispecific** antibody fragments for clinical applications. Carter P; Ridgway J; **Zhu Z.** (Department of Molecular Oncology, Genentech Inc., South San Francisco, CA 94080, USA.) JOURNAL OF HEMATOTHERAPY, (1995 Oct) 4 (5) 463-70. Ref: 63. Journal code: 9306048. ISSN: 1061-6128. Pub. country: United States. Language: English.

AB The clinical potential of **bispecific** antibodies (BsAb) has been hindered by the difficulty of obtaining clinical grade material, together with the immunogenicity of rodent-derived BsAb in patients. The supply issue is being directly addressed by recombinant methods for BsAb fragment production reviewed here. The immunogenicity issue will likely be overcome by the use of humanized or human antibodies. Currently, three technologies appear suitable for the production of BsAb fragments for clinical applications: BsF(ab')2 assembled from Fab' fragments expressed in Escherichia coli, BsF(ab')2 assembled using leucine zippers, and diabodies.

L33 ANSWER 16 OF 16 MEDLINE DUPLICATE 10
95355152 Document Number: 95355152. PubMed ID: 7628874. Engineering high affinity humanized anti-p185HER2/anti-CD3 **bispecific** F(ab')2 for efficient lysis of p185HER2 overexpressing tumor cells. **Zhu Z;** Lewis G D; Carter P. (Department of Cell Genetics, Genentech Inc., South San Francisco, CA 94080, USA.) INTERNATIONAL JOURNAL OF CANCER, (1995 Jul 28) 62 (3) 319-24. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB We previously constructed a humanized anti-p185HER2/anti-CD3 **bispecific** antibody variant, BsF(ab')2 v1 which retargets the cytotoxic activity of human T cells in vitro against human breast tumor cells which overexpress the p185HER2 product of the HER2/neu (c-erbB-2) protooncogene. Subsequently we identified an improved anti-CD3 variant, v9, which binds to T cells with approx. 100-fold higher affinity than the original variant, v1. Here we demonstrate that BsF(ab')2 v9 is more potent than BsF(ab')2 v1 in stimulating the proliferation of both resting peripheral blood lymphocytes (PBL) and IL-2-activated, long-term cultured T lymphocytes (ATL). In addition, at low concentrations (0.01-1 ng/ml) BsF(ab')2 v9 is much more efficient than BsF(ab')2 v1 in directing lysis of p185HER2-overexpressing tumor cells by IL-2 activated PBL. In contrast, at higher concentration BsF(ab')2 v9 and BsF(ab')2 v1 have similar potency in retargeted cytotoxicity. At BsF(ab')2 v9 concentrations of > or = 1 ng/ml the susceptibility of p185HER2-expressing tumor cells to lysis is apparently independent of the level of p185HER2 expression. At lower concentrations of BsF(ab')2 v9 and/or lower ratios of effector to target cells the extent of lysis is reduced, in some cases improving the selectivity of lysis of high p185HER2 expressors over low expressors. Thus selection of a high affinity anti-CD3 arm is likely important in the design of BsF(ab')2 for retargeting the cytotoxicity of T cells to tumors. The dose of BsF(ab')2 v9 in any future clinical evaluation will require optimization to maximize anti-tumor efficacy whilst minimizing potential toxicity against normal tissue expressing p185HER2.

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